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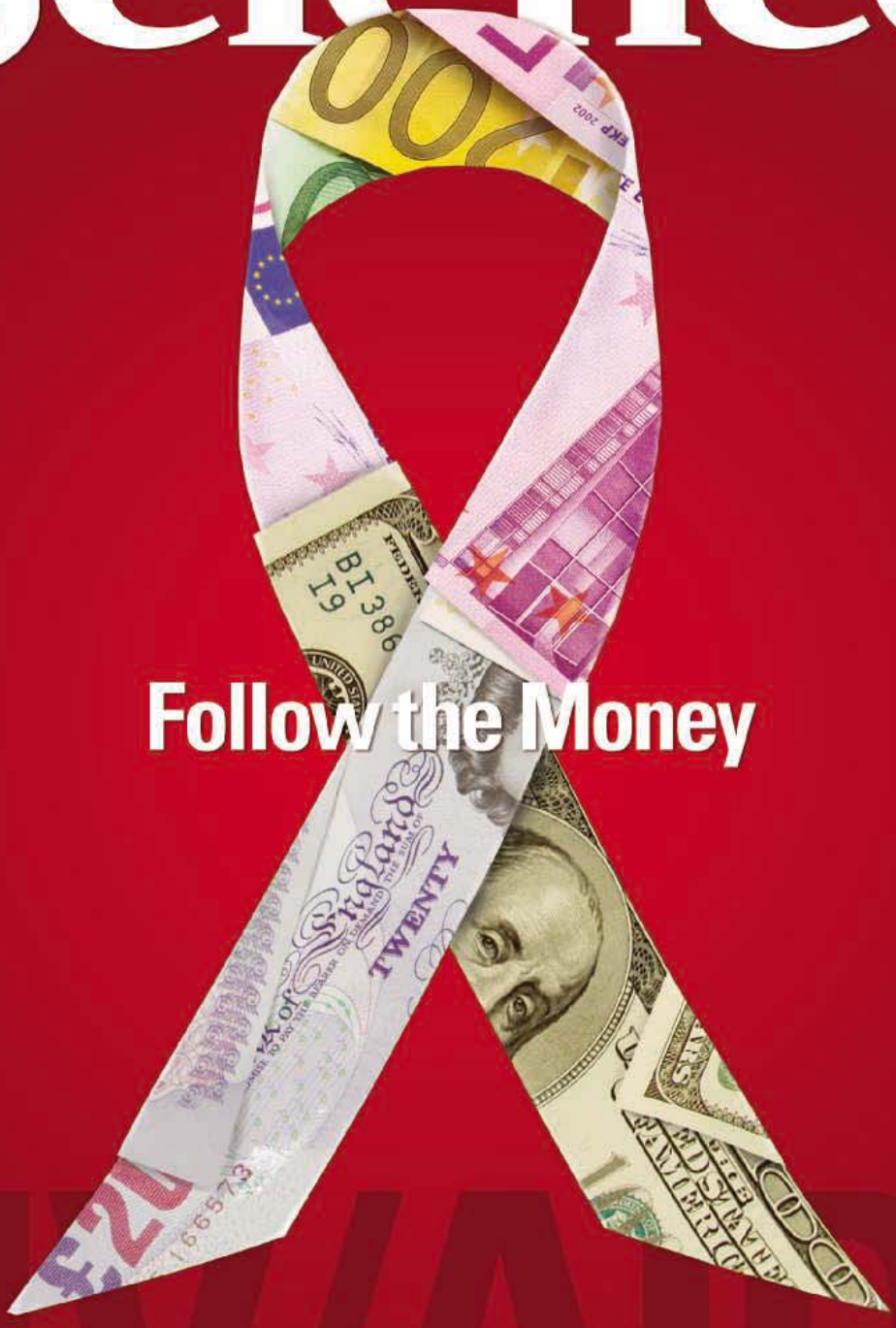

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HIV/AIDS

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COVER

Wealthy nations now devote billions of dollars each year to helping low- and middle-income countries confront HIV/AIDS epidemics. Investment in biomedical research has also shot up. What has come of this flood of money? And will there be enough in the future to meet increasing demands? See the special section beginning on page 511.

***Image: Kelly Krause/*Science**

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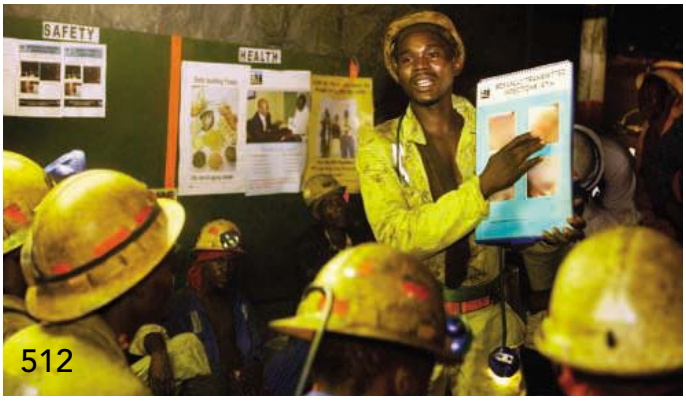

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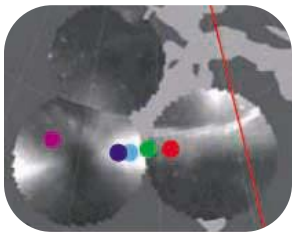
10.1126/science.1156121



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SCIENCE (ISSN 0036-8075) is published weekly on Friday, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue, NW, Washington, DC 20005. Periodicals Mail postage (publication No. 484460) paid at Washington, DC, and additional mailing offices. Domestic individual membership and subscription (51 issues): $144 ($74 allocated to subscription). Domestic institutional subscription (51 issues): $770; Foreign postage extra: Mexico, Caribbean (surface mail) $55; other countries (air assist delivery) $85. First class, airmail, student, and emeritus rates on request. Canadian rates with GST available upon request, GST #1254 88122. Publications Mail Agreement Number 1069624. **Printed in the U.S.A.**

Change of address: Allow 4 weeks, giving old and new addresses and 8-digit account number. **Postmaster:** Send change of address to AAAS, P.O. Box 96178, Washington, DC 20090–6178. **Single-copy sales:** $10.00 current issue, $15.00 back issue prepaid includes surface postage; bulk rates on request. The identification code for *Science* is 0036-8075. *Science* is indexed in the *Reader's Guide to Periodical Literature* and in several specialized indexes.

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C. elegans, the subject of WormBook.

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ST NETWATCH: WormBook
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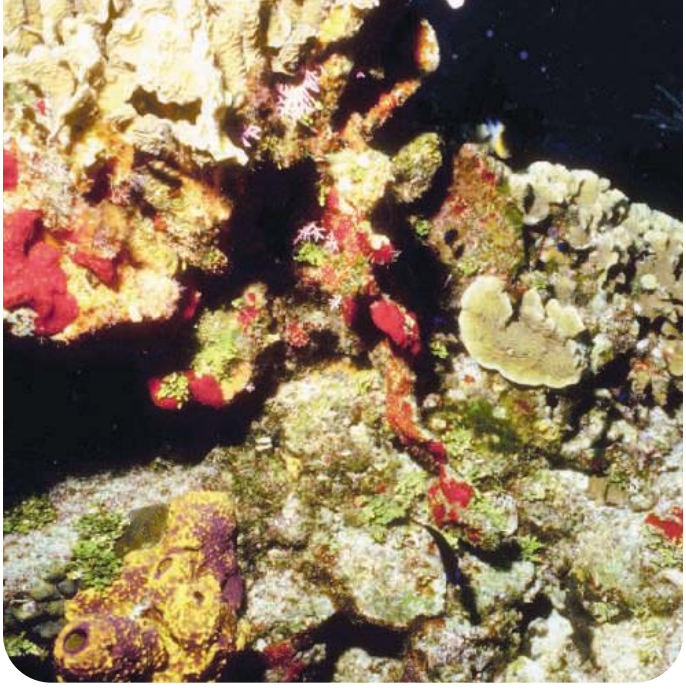


<< Coral Reefs Under Threat

The conservation status of coral reefs can be monitored by assessing the area covered by coral species over time. **Carpenter *et al.*** (p. 560, published online 10 July) have estimated that more than a third of the major reef-building coral species are at risk of dying out to the point at which reef viability is lost. The causes of this dismaying decline stem from local insults from physical damage, overfishing, pollution, and sedimentation. These factors, added to the physiological harm done to coral organisms and their symbionts by elevated sea surface temperature rise and water acidification induced by atmospheric greenhouse gas accumulation, can mean that a reef loses viability and quickly turns into a mound of rubble.

Aberration-Corrected Electron Microscopy

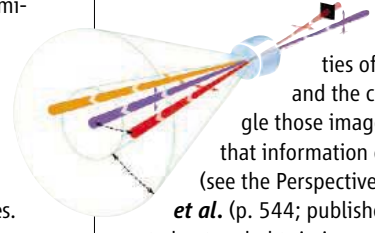
Transmission electron microscopes have been used for a long time to study the structures of materials, but aberrations introduced by the electron optics of the instruments have limited their spatial resolution. **Urban** (p. 506) reviews recent advances in developing instruments that are largely free of aberrations. These aberration-corrected electron microscopes allow atomic positions to be determined with unprecedented accuracy. Furthermore, they enable determination of the occupancies of atom sites and atomic-scale imaging of chemical composition and bonding. The instruments have been used to study, for example, twin-boundary structures, sublattice structures in multilayered materials, and the atom arrangements in catalyst particles.

Small RNAs and the Argonautes

Small RNAs play a critical role in regulating a wide range of cellular processes in eukaryotes, the most thoroughly characterized of which occur in the cytoplasm. Small RNAs also function in a number of processes in the cell nucleus, including heterochromatin formation and genome rearrangement. **Guang *et al.*** (p. 537; see the Perspective by **Meister**) describe a genetic screen in the nematode *Caenorhabditis elegans* for factors required for nuclear RNA interference. The findings suggest that an Argonaute-like protein, NRDE-3, which contains a nuclear localization signal, is involved in many RNA-based nuclear silencing processes. Furthermore, the nuclear localization signal and the binding of small interfering RNAs generated by the action of RNA-dependent RNA polymerases on messenger RNAs in the cytoplasm are required for NRDE-3 to relocate from the cytoplasm to the nucleus.

A Picture Speaks a Thousand Words

There is a lot of information contained in images, and this complexity can be exploited, for example, in security-key generation and data encryption. Using the quantum properties of light in images, and the capability to entangle those images, would multiply that information capacity many-fold (see the Perspective by **Boyd**). **Boyer *et al.*** (p. 544; published online 12 June) created entangled twin images by passing the light through a cloud of warm rubidium atoms. In the likes of optical tweezers or large-array interferometers, precision measurements are based on monitoring the position of laser beams. Entanglement can also be used to improve measurement. In pursuit of this goal, **Wagner *et al.*** (p. 541) were able to entangle two lasers, which could now potentially be applied to enhancing spatial measurements.



Phase Transitions in Bismuth

Recent work on graphene, a single-layered sheet of carbon atoms in which the electrons move at constant velocity that can be described in terms of relativistic behavior, has triggered interest in other materials that exhibit "Dirac fermion" behavior. Bismuth is of particular interest because its band structure is 3-dimensional and its electrons are also Dirac fermions. As such, exotic electronic phase transition might be expected. By applying high magnetic field at low temperature, **Li *et al.*** (p. 547; see the Perspective by **Behnia**) take bismuth to the quantum limit and observe a novel collective electronic phase in which the pockets of electrons that make up the energy bands align with ferromagnetic ordering.

Birth of the Cool

Biodiversity during the Ordovician Period (between 490 and 440 million years ago) increased tremendously, in perhaps the greatest evolutionary radiation in Earth's history. Why did this explosion of variety occur? **Trotter *et al.*** (p. 550) present a record of sea surface temperatures for the period that shows that the radiation took place only when temperatures had decreased from values as high as 10°C higher to ones similar to modern times. This cooling, which occurred at a fairly uniform pace over the first 25 million years of the Ordovician, was estimated by analyzing the oxygen isotope composition of the calcium phosphate mineral apatite. The data suggest significantly cooler conditions than those derived from older records based on carbonates, bringing estimates more into line with what is understood about the fossil record.

Increasing Thermopower Through Scattering

Thermoelectric generators and coolers are formed from junctions of semiconductor materials that are

Continued on page 463

CREDITS (TOP TO BOTTOM): PHOTOS.COM; BOYER *ET AL.*

Continued from page 461

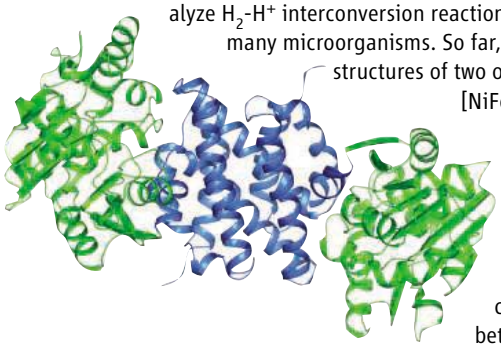
good electrical conductors but poor thermal conductors. Efforts to improve these materials have focused mainly on decreasing their thermal conductivity by increasing phonon scattering, but this approach reaches an ultimate limit, that of the amorphous material. However, the figure of merit that describes these materials, *ZT*, also depends on the thermopower, or Seebeck coefficient *S*, and theory suggests that *S* can be increased by an appropriate increase in the density of states of the material. **Heremans *et al.*** (p. 554) report that thallium doping of lead telluride, a commonly used thermoelectric, can improve its *ZT* by about 50% at temperatures of ~750 kelvin.

Brassinosteroid Signaling

Brassinosteroids, a type of steroid hormone in plants, regulate a variety of developmental processes. Some genetic targets and signaling components targeted by the brassinosteroids have been identified, but the full chain of command remains unclear. By focusing their attention on proteins in the plasma membrane or proteins modified by phosphorylation, **Tang *et al.*** (p. 557) were able to identify two previously unknown kinases (BRK1 and BRK2) that interact with the brassinosteroid receptor at the plasma membrane. BRI1 kinase, the brassinosteroid receptor, phosphorylates these two substrate kinases to activate downstream brassinosteroid signaling.

Crystal Clear Hydrogenation

The ability to catalyze dissociation of hydrogen efficiently is of great interest in alternative energy technology. One approach is to develop catalysts that mimic the hydrogenase enzymes that catalyze H_2-H^+ interconversion reactions important in the energy metabolism of many microorganisms. So far, these efforts have been guided by the structures of two of the three classes of hydrogenases: [NiFe]-hydrogenase and [FeFe]-hydrogenase. Now **Shima *et al.*** (p. 572; see the Perspective by **Armstrong and Fontecilla-Camps**) describe the structure of the third class, Fe-hydrogenase, in which a mononuclear iron is part of an iron guanylyl pyridine cofactor without an Fe-S cluster. There is surprising similarity between the active sites of the three enzymes. The convergent features are likely to play an important role in hydrogenation and may provide a clearer framework for the design of new catalysts.



CREDIT: SHIMA *ET AL.*

Dissecting a Garbage Disposal Mechanism

When secretory and membrane proteins misfold in the endoplasmic reticulum (ER), an important mechanism for their disposal, ER-associated degradation (ERAD), is put in train. Misfolded proteins are retrotranslocated into the cytosol, where they are degraded by the ubiquitin-proteasome system. The oxidative environment in the ER generates disulfide bond–mediated high-molecular-weight complexes from misfolded proteins that must be reduced prior to their retrotranslocation during ERAD. **Ushioda *et al.*** (p. 569; see the Perspective by **Braakman and Otsu**) have now identified ERdj5 as a disulfide reductase in the ER. ERdj5 accelerates ERAD by cleaving intermolecular disulfide bonds of misfolded proteins. ERdj5 associates with EDEM, a lectin-like ERAD protein that recognizes misfolded proteins to be degraded. ERdj5 also associates with BiP, an ER molecular chaperone.

Manipulating Mitochondria

Mitochondria, the metabolic powerhouse of the cell, are relic bacterial symbionts that contain their own vestigial genomic DNA, mitochondrial DNA (mtDNA). Mutations in mtDNA-encoded genes can result in serious cellular dysfunction and disease. MtDNA is present in many copies and inherited in a non-Mendelian manner, making study of defects in mitochondrial metabolism difficult. To address this problem, **Xu *et al.*** (p. 575) have developed a system for importing restriction enzymes into the mitochondria of *Drosophila* germ cells and selecting for embryos containing within their mitochondria mutations in cognate restriction enzyme target sites. Mutations in the cytochrome C oxidase gene, for example, show a range of phenotypes, including male sterility, age-dependent neurodegeneration, and myopathy, similar to those caused by mutations in human mtDNA.

Luis E. Soto-Ramírez is a researcher and head of Molecular Virology at the Department of Infectious Diseases of the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán in Mexico City. He is also co-chair of the XVII International AIDS Conference. E-mail: lsoto@quetzal.innsz.mx

HIV/AIDS in Latin America

SIXTEEN INTERNATIONAL AIDS CONFERENCES WERE HELD BEFORE THE HIV/AIDS COMMUNITY looked to Latin America and the Caribbean. Finally, next month, the world will pay long-overdue attention to this region when the XVII International AIDS Conference (AIDS 2008) is held in Mexico City. Given that so many in Latin America and the Caribbean have died from HIV/AIDS and have fought this disease with the same enthusiasm and passion as in any part of Africa and Asia, there seems no justification for this lack of attention.

Although no country in Latin America and the Caribbean has the double-digit HIV prevalence seen in sub-Saharan Africa, there are an estimated 2 million people living with HIV/AIDS in the region, with about 120,000 new cases and 70,000 deaths in 2007. The cumulative estimate for 2015 is nearly 3.5 million HIV/AIDS cases and 1.5 million deaths from the disease.

There are issues in Latin America and the Caribbean that make epidemic conditions unique to the region. Many people still do not understand that HIV/AIDS is a viral, not a moral, infection. Widespread stigma and discrimination hamper efforts to achieve universal access to HIV prevention, treatment, and care. HIV transmission continues to occur among populations at higher risk, including sex workers, males that have sex with males (but increasing among heterosexuals), injecting drug users, and migrants. Prevention efforts, including education campaigns, are disorganized and poorly supported because budgets are mainly devoted to treatment.

As far as treatment is concerned, for the past 5 to 10 years, most governments in Latin America and the Caribbean have been trying to provide antiretroviral drugs to all in need. This strategy is thought to be the most cost-effective, as well as the most visible, and is therefore the primary goal of most countries in the region. However, some countries that implement wide access to treatment have forgotten that universal access to expert care, prevention, and the monitoring of laboratories are as important as access to drugs. The education of more physicians to treat the disease has become a major challenge because many specialists do not want to treat HIV-infected individuals because of stigma and the fear of contracting AIDS themselves. Providing adequate care is even more complicated because tests that monitor response to treatment are not always given by the health care systems. Even when such tests are available, they are performed every 6 or more months apart (rather than every 3 to 4 months). This lack of monitoring allows more patients to develop viral resistance to drugs, and this can lead to a decrease in the efficacy of subsequent antiretroviral treatments, which are more expensive. The problem is compounded by the fact that infected people usually don't seek treatment until they have symptoms and/or low T cell counts. Together, these factors have decreased the impact of treatment on mortality reduction. As a consequence, HIV/AIDS is taking its toll on health expenditures in the region and on lives. These are not unlike problems experienced elsewhere, so there are opportunities to help countries in Latin America and the Caribbean avert the projected devastating effects the disease will have during the next decade.

But not all is bad. At the AIDS 2008 conference, Latin America and the Caribbean will showcase good examples of how to provide universal antiretroviral treatment with and without generic drugs, and highlight extraordinary activism among communities, governments, religious organizations, and researchers. These groups are working together to thwart the epidemic through new programs, including group-specific education campaigns and efforts to reach migrants as well as rural populations that sometimes speak local dialects.

The theme of AIDS 2008 is Universal Action *Now*, implying that immediate actions are needed in all regions of the world suffering from HIV/AIDS. By understanding the common and different issues across countries that are related to overcoming the disease, we can hopefully promote the most effective actions possible for each region, for each country, and for each human being.

– Luis E. Soto-Ramírez

10.1126/science.1162896

CREDITS: GABRIEL GONZALEZ, MALCOLM LINTON

VIROLOGY

Modeling Microbicides

Phase III clinical trials are underway to test the efficacy of antiretroviral (ARV)–based microbicides in preventing HIV transmission. However, the risk that ARVs may be absorbed systemically and promote the evolution of drug-resistant viral strains if used by HIV-positive women remains poorly characterized.

Wilson *et al.* have modeled the effects of ARV-based microbicides on disease dynamics, either as part of a clinical trial or as a widespread public health intervention, in order to compare drugs with a high versus low potential risk for generating resistance. They find that a clinical trial will be unable to distinguish between high- and low-risk microbicides if HIV-positive participants are excluded on the basis of monthly tests for seroconversion (as planned for the upcoming dapivirine trial), given that resistance is expected to take at least 6 months on average to develop. If a high-risk microbicide is used as a public health intervention, the model predicts that the ratio of the number of prevented infections to the number of acquired resistant cases (the benefit-to-cost ratio) may not be much greater than 1. The ratio will be worse for women than for men, given that new cases of resistance will emerge in women initially and that drug-resistant strains have lower transmission efficiency than wild-type HIV. These results highlight the importance of collecting additional data on the resistance risks of new ARV-based microbicides before they are approved for popular use. — NM*

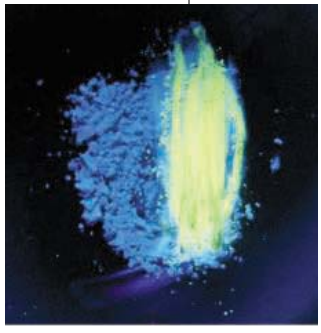
Proc. Natl. Acad. Sci. U.S.A. **105**, 9835 (2008).

*Nilah Monnier is a summer intern in *Science*'s editorial department.

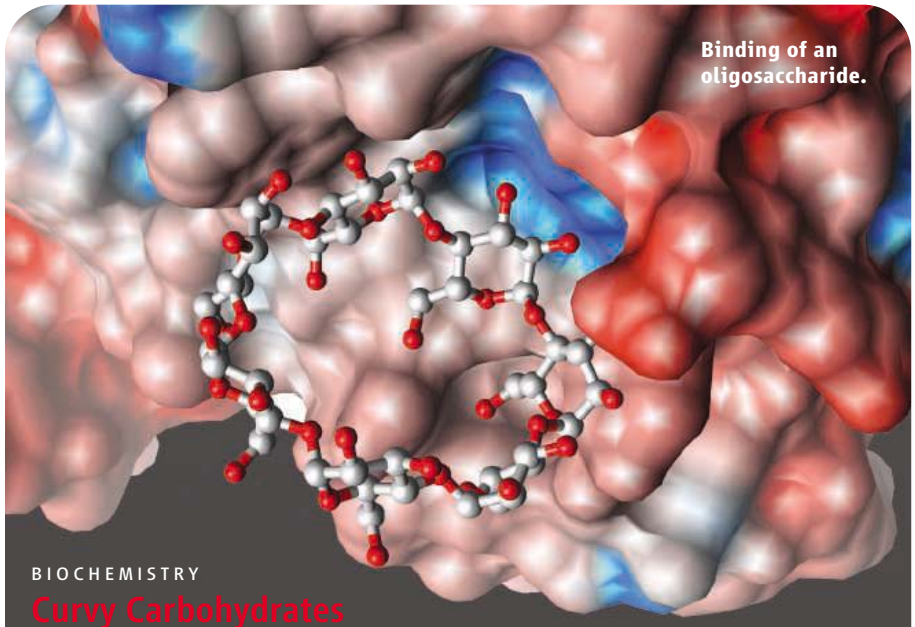
CHEMISTRY

Blue, Yellow, and Gold

Mechanochromic compounds, which undergo a change in color or luminescence when solid samples are crushed or ground, can serve as detectors of mechanical action, but examples of such compounds are rare. Ito *et al.* synthesized a compound in which two C_6F_5Au groups are linked by a *para* $CN(C_6H_4)NC$ ligand, and found that its photoluminescence changes from blue to yellow after grinding. Like other such compounds, its original luminescent state is restored upon dissolution and recrystallization, and this process could be repeated for 20 cycles without any decrease in luminescence. Structural and spectroscopic studies indicate that the long-lived blue emission in the crystal is intramolecular in origin and phosphorescent (a localized intraligand π–π^* transition), whereas the yellow emission appears to arise from an amorphous phase characterized by aurophilic interactions: intermolecular interactions between gold atoms. — PDS



J. Am. Chem. Soc. **130**, 10.1021/ja8019356 (2008).



Binding of an oligosaccharide.

BIOCHEMISTRY

Curvy Carbohydrates

The global abundance of carbohydrate-based biopolymers and the prevalence of them in our diets support within the human gut a fascinating microbial ecosystem. Its role, from our point of view, is to degrade dietary polysaccharides; the flux through these pathways contributes as much as 10% of our daily calorie intake. One of the inhabitants of the human intestine is *Bacteroides*, and its starch utilization system (Sus) contains a number of regulatory and metabolic genes.

Koropatkin *et al.* have determined the structure of the outer membrane protein SusD on its own and with linear and cyclic oligosaccharides bound. They find that the side chains of aromatic residues of SusD align to offer a curved surface that complements the helical conformation of maltoheptaose as well as the curvatures of α- and β-cyclodextrins (composed of six and seven glucose units, respectively). The multivalent, low-affinity interaction may facilitate hydrolysis of longer polysaccharides by the neighboring amylase SusG or the loading of oligosaccharides into the outer membrane importer SusC. — GJC

Structure **16**, 1105 (2008).

APPLIED PHYSICS

Unscintillating Conversation

Air-to-air and air-to-ground communication are generally carried out with radiofrequency transmitters and receivers. By comparison, optical-based communication systems offer a number of advantages, including higher bandwidth and the capacity to communicate information at higher rates, bundled with low weight, compact size, low power requirements, and the added security of a direct line-of-sight communication channel. However, the air turbulence that makes stars twinkle or scintillate at a detector presents a substantial problem for airborne optical communication. Unchecked, such turbulence will introduce a large amount of error into a communication channel. As an alternative to the relatively large adaptive-

CREDITS (TOP TO BOTTOM): TOM SMITH; ITO *ET AL.*, *J. AM. CHEM. SOC.* **130**, 10.1021/JA8019356 (2008)

optics approach that uses a reference beam to remove the twinkle from the stars with wavefront engineering, Louthain and Schmidt show that a multiple-transmitter approach using several optical beams can also work. Their numerical simulations take into account a number of factors such as beam separation, phase difference, and angle of propagation of the individual beams. By averaging out the contribution from turbulence-induced shifts they show that the multiple-beam approach can significantly reduce the bit-error rate in messages over the optical channel. — ISO

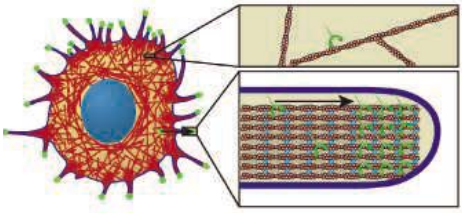
Opt. Express **16**, 10769 (2008).

CELL BIOLOGY

Moving Through a Crowd

A migrating eukaryotic cell has a dense mesh of cortical actin at its leading edge, with long parallel actin bundles extending into microspikes and filapodia. Myosin X localizes at the ends of filapodia, where it may be involved in processes such as adhesion and signaling. How does myosin X find the appropriate actin filaments and travel along them to reach its destination?

Nagy *et al.* show that although myosin X processive runs on single filaments are short and rare, it moves robustly and at length on fascin-bundled actin, which makes up the core of filapodia. Myosin X has a short neck so that its step size is probably smaller than the actin pseudo-helical repeat, which might account for its low processivity on single filaments. On actin bundles it can move with one head tracking one filament and the other head on the adjacent filament. Furthermore, myosin X was



Schematic of myosin X (green), fascin (blue), and actin filaments (red) in a filapodium.

observed to move even farther and faster on artificially bundled (by molecular crowding) actin, suggesting that filament proximity facilitates its movement rather than structural features specific to actin monofilaments. — VV

Proc. Natl. Acad. Sci. U.S.A. **105**, 9616 (2008).

BIOMEDICINE

Editing T Cells

The chemokine receptor CCR5 is one of the major docking sites that HIV uses to enter T cells, and individuals carrying a homozygous deletion mutation in *CCR5* are resistant to HIV infection. Hence, interfering with the HIV-CCR5 interaction has become the goal of several small-molecule drug design projects.

Perez *et al.* have instead taken the approach of disrupting *CCR5* by introducing a double-stranded break into the gene itself. Zinc-finger nucleases were designed to bind to and cleave at specific sequences found only in *CCR5*; endogenous DNA repair pathways have the effect of introducing deletions or insertions that yield a truncated or nonfunctional protein. Cultured human T cells that were transduced with a vector expressing the zinc-finger nucleases were resistant to HIV infection, and long-term expansion of these cells in culture did not reduce their resistance. In a mouse model of acute HIV infection, animals treated with nuclease-modified human T cells had a lower plasma viral load than control mice, along with higher T cell counts, suggesting that genome editing might be used therapeutically to provide a pool of HIV-resistant T cells. — LC

Nat. Biotechnol. **26**, 808 (2008).

PHYSIOLOGY

Fast Rising Hormone

Fibroblast growth factor 21 (FGF21) has recently taken center stage as a key metabolic hormone that helps the body to adapt to starvation. When mice are fasted, FGF21 expression levels in the liver rise dramatically. In turn, FGF21 stimulates the release of fatty acids from adipose tissue and promotes their conversion in the liver to ketone bodies, which can be used as an energy source when carbohydrates are scarce; FGF21 also promotes torpor, an energy-conserving state in mice characterized by a reduction in body temperature and physical activity.

Inagaki *et al*. show that the role of FGF21 in programming energy conservation during starvation may be even broader. Through the use of transgenic mice, they found that FGF21 mimics the inhibitory effects of fasting on organismal growth at both the phenotypic and molecular levels. The transgenic mice were smaller than their wild-type counterparts despite equal or greater food intake. Although the transgenic mice had higher levels of circulating growth hormone (GH) than controls, they appeared to be resistant to its actions. In the liver, FGF21 reduced the expression of a major mediator of GH action—the transcriptional regulator STAT5—and decreased the expression of key target genes implicated in organismal growth, including the gene encoding insulin-like growth factor 1 (IGF-1). Whether FGF21 has similar effects in humans remains to be determined. — PAK

Cell Metab. **8**, 77 (2008).

CREDIT: NAGY *ET AL.*, *PROC. NATL. ACAD. SCI. U.S.A.* **105**, 9616 (2008)

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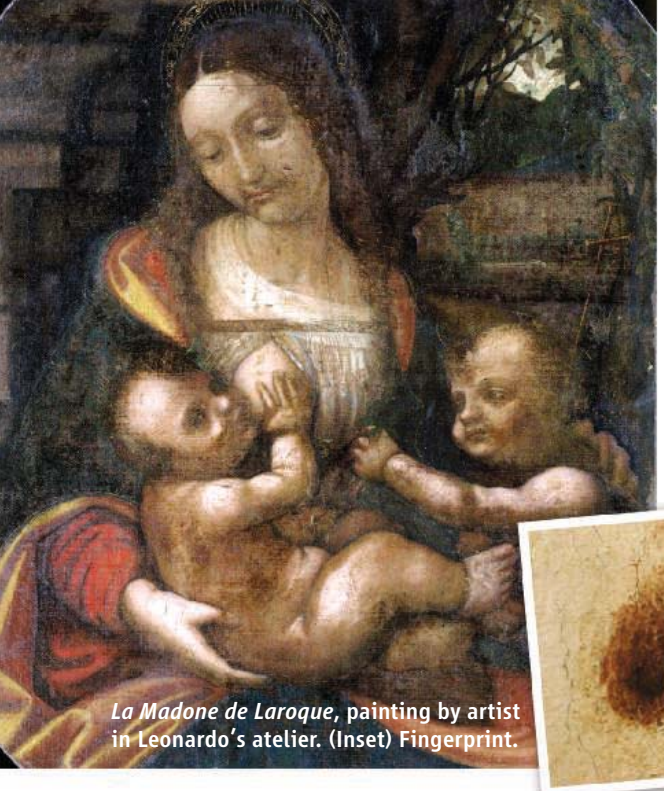
Information for Authors
See pages 634 and 635 of the 1 February 2008 issue or access www.sciencemag.org/about/authors

The Master's Touch



La Madone de Laroque, painting by artist in Leonardo's atelier. (Inset) Fingerprint.

Fingerprints on a painting from the studio of Leonardo da Vinci show the touch of the master himself—and confirm that the artist had Arab ancestors, Italian researchers say.

A team led by Luigi Capasso, an anthropologist at the Museum of Biomedical Sciences in Chieti, Italy, is using infrared light to study prints on *The Martyrdom of Saint Catherine* and *La Madone de Laroque*—paintings attributed to members of Leonardo's atelier in Amboise, France. The artist often used his fingers in place of brushes, diluting colors with saliva. But experts couldn't tell whether any of the fingerprints on the paintings were his.

The researchers have now matched one of the prints to a fingerprint on *Lady with an Ermine*, known to be by Leonardo. Some scholars say Leonardo's mother was an Arab slave, and Capasso and colleagues at the Museo Ideale in Vinci says their research confirms the artist's Middle Eastern origins. The print, from his left index finger, has a Y-shaped pattern shared by 60% of Middle Easterners, says Emiliano Carnieri, a paleontologist at the University of Palermo in Italy. Fingerprints, like blood group or skin color, can help determine a person's ancestral origins, Carnieri says.

Alessandro Vezzosi, director of the Museo Ideale, calls the research "extraordinary and very important" in casting light on techniques of Leonardo and his followers.

Wolves Wanted

Gray wolves last roamed the rainforests of Olympic National Park in Washington state in the early 1900s. And streamside forests, preserved in 1938 as "the finest example of primeval" woods in the United States, have gone downhill ever since, say scientists at Oregon State University, Corvallis.

Without wolves, elk feast at will on trees and shrubs along rivers and streams, leading to severe erosion. "The rivers [in the park] are dramatically degraded," says hydrologist Robert Beschta, co-author of a study published online this month in *Ecohydrology*. He says historical records describe the banks of the upper Quinault River as "so dense with underbrush as to be almost impenetrable." Today, the same areas are open glades dominated by big trees. "There has been almost no recruitment of new cottonwood and bigleaf maple trees"—species elk find especially tasty—since the wolves were extirpated, adds ecologist William Ripple. The team has found similar problems in other parks missing their top predators (*Science*, 2 May, p. 597; 27 July 2007, p. 438).

Experts agree that the Olympic ecosystem needs wolves, and "wolves are on our list to consider" for reintroduction, says the park's lead wildlife biologist, Patti Happe. "But it will take a lot of public support to happen." Plans to reintroduce the species in the 1990s were shelved due to local resistance.

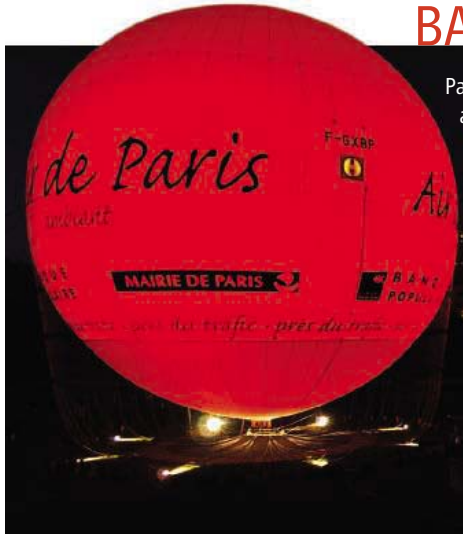
Pole to Pole

On 14 July, the world got just a little bit smaller, with the first satellite communications link between the north and south poles.

Scientists at the Airship Italia Arctic research station on the Svalbard Islands near the North Pole established an audio and video link with colleagues at the Concordia base in Antarctica. The event marked the 80th anniversary of the fatal flight of Italia, an airship built by Arctic explorer Umberto Nobile, which crashed while returning to Norway from the North Pole. Nobile and seven others were picked up after weeks on floating ice, but the Norwegian explorer Roald Amundsen died in the rescue efforts.

The link underscored the connection between Arctic and Antarctic research, says geoscientist Giuseppe Cavarretta of Italy's National Research Council in Rome. Last week, workers in Svalbard also laid the first stone for a new 35-meter-high research tower that will measure temperature, air composition, and other atmospheric parameters. The €50,000 Amundsen-Nobile tower is a near-duplicate of an American tower in the Antarctic that has been gathering data since 2002. "This time we are not 'exploring' with an airship but with sophisticated scientific instruments," Cavarretta says.

BAD AIR DAY?



Parisians living near the Seine can now gauge local air quality simply by looking out the window. A helium balloon tethered in a park by the river was recently equipped with lasers that, at night, make it glow in one of five colors ranging from green (signaling very good air) to red (very bad). The color follows an index that factors in nitrogen dioxide, ozone, and particle levels around the city.

During the day, when the laser can't be seen, a colored canvas wrapped around the gondola and a flag convey the information. As of September, a separate spot on the balloon's surface will also show average air quality near major Parisian traffic junctions.

CREDITS (TOP TO BOTTOM): AFP/GETTY IMAGES; MUSEO IDEALE; JULIE LAWRENCE, WOLF HAVEN INTERNATIONAL; BALLON AIR DE PARIS

New Products, Services, and Solutions

EDITED BY YUDHIJIT BHATTACHARJEE

Pioneers

MINUS THE SCOTCH. A University of California (UC), Davis, English professor who studies literature about the environment went totally green for his talk at this year's annual meeting of the Association for the Study of Literature and Environment. Instead of flying to Edinburgh, U.K., for the 10 to 13 July conference, Timothy Morton mailed in a DVD of his lecture and took questions from the audience via videoconference after it was shown.

"New technology gives us a chance to save energy and save money and reimagine in a good way what a conference could be," says Morton, who's pressing UC administrators to create a technological infrastructure—à la the online community Second Life—to support virtual conferencing.

Morton thinks the discussion after his video speech on the implications of nature writing for cognitive science suffered not at all from his being thousands of miles away from the audience. But he did miss swapping gossip with colleagues during the meeting. And for the record, he says he would have done the same thing had the conference been in the French Riviera.

THEY SAID IT

"A sick patient does not represent a biochemistry problem, an anatomy problem, a genetics problem, or an immunology problem."

—Jules Dienstag, dean for medical education at Harvard Medical School, making the case for reforming the premed curriculum to focus it more narrowly on human biology and clinically relevant subjects, in an essay in last week's *New England Journal of Medicine*.

IN BRIEF

James Briscoe of the United Kingdom's National Institute for Medical Research has been awarded the European Molecular Biology Organization's Gold Medal for his research on the molecular basis of neuronal development in the spinal cord.

UPDATE

German neuroscientist **Tobias Bonhoeffer** has declined an offer to become the first president of the Institute of Science and Technology Austria. In a 21 July statement, the institute said that Bonhoeffer told the heads of the search committee and the institute's governing board that personal reasons and "a desire to continue his research in his current position" led him to this "difficult decision." Bonhoeffer, now at the Max Planck Institute of Neurobiology, declined comment. The institute will reopen its search and hopes to have a new president in place by autumn 2009.

A Three Q's with Bonhoeffer in last week's Newsmakers page (*Science*, 18 July, p. 323) failed to note that he had not yet made a decision.

Slice of Life >>

LIVING OFF THE SUN. Henry Kelly is coming up on an expensive ritual he has to carry out every 8 years: replacing the $1000 batteries in his solar-powered country home near Culpeper, Virginia. Since building the house 25 years ago, the physicist, who heads the Federation of American Scientists, has learned firsthand the joys and annoyances of living off an array of solar panels, at least on weekends. "It feels great to know that your word processing, for example, has been powered by the sun," says Kelly, who's advocated for the expansion of alternative energy and energy-efficiency technology.

But his experience has also shown him the technical limitations of solar technology as well as some frustrating aspects of the off-the-grid lifestyle. The only viable storage technology available remains the lead nickel batteries he originally installed. The power needed to run the drinking water pump "tends to kill the charge" in the batteries, which means dry pipes at night, and the system hums so loudly that he had to build it a special shed. His family has also become more conscious of conserving energy there—"you are very aware of not plugging in hairdryers, turning lights off," he says—although his 24-year-old daughter usually drains the battery during New Year's Eve parties.

CREDITS (TOP TO BOTTOM): SOURCE: TIMOTHY MORTON; MONICA AMARELO; FAS

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AIDS VACCINE RESEARCH

Thumbs Down on Expensive, Hotly Debated Trial of NIH AIDS Vaccine

When he scuttled plans for a huge AIDS vaccine efficacy trial last week, Anthony Fauci, director of the U.S. National Institute of Allergy and Infectious Diseases (NIAID), erased any doubts that he is willing to buck his own advisers when necessary. Fauci made his decision even though NIAID's AIDS Vaccine Research Subcommittee (AVRS) voted in May in favor of a study of a candidate designed by scientists at his own institute. "Voting is important provided you have the opportunity to ask people why did you vote that way," says Fauci, who spoke with several subcommittee members after the meeting. "When you ask [them] for the scientific rationale, they often change their minds."

In canceling the Partnership for AIDS Vaccine Evaluation (PAVE) study, which would have cost $63 million, Fauci also challenged researchers to come up with a "lean and mean" alternative. Fauci, who lately has faced intense pressure from AIDS vaccine investigators to put more money into fundamental research (see p. 530), says so much confusion exists about what a vaccine should contain that proceeding with this particular vaccine was simply too dicey. "Given the fuzziness of all this, I'm just not willing to go ahead with such an expensive trial." Fauci encouraged researchers to design a smaller, cheaper clinical trial that would reveal whether the vaccine has sufficient promise to warrant a larger efficacy study.

The PAVE trial had already been dramatically scaled back from the initial plans for this vaccine, which is made by NIAID's Vaccine Research Center (VRC). Last fall, NIAID had hoped to start testing the vaccine in 8500 people around the world, for an estimated $140 million. But just when the trial was about to start, evidence surfaced that a similar AIDS vaccine made by Merck & Co. did not work and may even have left some people more vulnerable to HIV infection (*Science*, 16 November 2007, p. 1048). NIAID promptly canceled that trial and began reassessing plans for the PAVE study.

Both the Merck and VRC vaccines use adenovirus-5 (Ad5), a type of cold virus, to deliver HIV genes into human cells. In the Merck study, for some unknown reason, the vaccine led to higher infection rates in uncircumcised men who had high antibody levels to Ad5 before the study began. Leaders of the PAVE study redesigned their trial to include only 2400 circumcised men who had low levels of Ad5 antibody. But Fauci said he worried that this study would ultimately not have the power to determine which immune responses correlate with protection, the central mystery in the frustrating search for an AIDS vaccine.

Buck stopper. NIAID's Anthony Fauci nixed an expensive study—overruling advisers.

AVRS member Dennis Burton applauds the decision. "The vaccine was too similar to the failed Merck vaccine to warrant a large-scale trial," says Burton, an immunologist at the Scripps Research Institute in San Diego, California.

NIAID's Gary Nabel, who designed the VRC vaccine—which contains more genes than the Merck vaccine and uses a priming dose delivered by a naked DNA plasmid—stresses that this is not the end of the project. "What's been proposed is to try and reach a middle ground where we can still move forward and learn," says Nabel. Fauci says he would like to see a smaller trial evaluate whether people who receive the vaccine and later become infected with HIV better control the virus. "We're not going to blow $63 million right off the bat until we get a signal that it's working," says Fauci. Two smaller trials of the VRC vaccine currently under way (see table) may also yield some insights.

Even AVRS members who supported the scaled-back study were relatively sanguine. "I'm a little disappointed," says Louis Picker, who studies AIDS vaccines at Oregon Health & Science University in Portland. "We could learn an amazing amount by establishing immune responses in people and following them both in terms of if they get infected and if they do not." Then again, Picker agrees that the VRC vaccine is far from ideal. "Everyone in their hearts is disappointed we don't have something better," says Picker.

Fauci says the money not spent on the PAVE study will become available to fund basic research, but he cautions that even a lean-and-mean trial of the vaccine could still cost $45 million. **–JON COHEN**

IN THE PIPELINE

Vaccine	Sponsor/Developer	Size	Trial sites	Start
PHASE III				
Canarypox-eng-gag-pol, gp120 boost	U.S. military, Thai Ministry Pub Hlth, Aventis, Vaxgen	16,402	Thailand	Oct. '03
PHASE II				
5 lipopeptides (gag, nef, pol)	ANRS, Aventis	132	France	Sep. '04
DNA-gag-pol-env/ Ad5-gag-pol-env boost	VRC, NIH, Vical, GenVec	480	U.S., Brazil, South Africa, Haiti, Jamaica	Sep. '05
Adeno-associated virus (type2)-gag-pol-RT	Targeted Genetics, Children's Hosp. of Penn., Indian Council of Med. Res./Natl. AIDS Ctrl. Org.	91	South Africa, Uganda, Zambia	Nov. '05
PHASE I/II				
DNA-gag-pol-env/ Ad5-gag-pol-env boost	VRC, NIH, U.S. military	324	Kenya, Uganda, Tanzania	May '06
DNA-env-gag-rev-RT/ MVA-eng-gag-pol boost	Muhimbili U., Karolinska, SMI, Vecura, U.S. military	60	Tanzania	Dec. '06
DNA-env-gag-pol-nef/ NYVAC-env-gag-pol-nef boost	European Comm., ANRS	140	U.K., Germany, Switzerland, France	June '07
DNA-gag-pol-env-vpu-tat-rev/ fowlpox-gag-pol boost	U. New South Wales, HIV Netherlands, Australia, Thailand Res. Collab.	24	Thailand	May '07

CREDITS (TOP TO BOTTOM): NIAID; SOURCE: NIH/IAVI/AVAC

Will solar telescope see the light?
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Pioneers of the science earmark era
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SCIENTIFIC MISCONDUCT

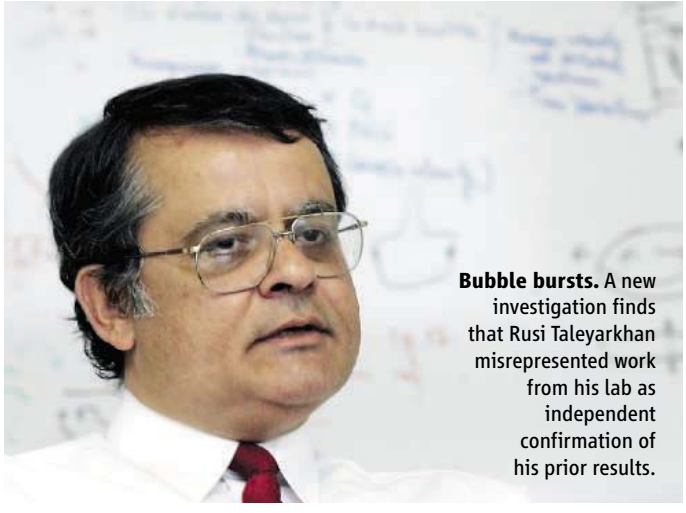
New Purdue Panel Faults Bubble Fusion Pioneer

The third time was no charm for Rusi Taleyarkhan, the "bubble fusion" pioneer at Purdue University in West Lafayette, Indiana. After two previous investigations looked into alleged scientific misconduct by Taleyarkhan, a third panel has now cited Taleyarkhan for two cases of misconduct. Both cases centered on efforts by Taleyarkhan to make experiments carried out by members of his lab appear as independent verification of his previous work.

Taleyarkhan first sparked controversy after he and colleagues reported in *Science* in 2002 that they had generated nuclear fusion with a simple tabletop setup. Fusion, the process that powers the sun, normally takes place at pressures and temperatures intense enough to cause atomic nuclei to combine and give off energy in the process. Decades' worth of efforts to harvest energy from that process in reactors on Earth have failed. In their original *Science* paper, Taleyarkhan, who was then at Oak Ridge National Laboratory in Tennessee, and his colleagues reported that firing a pulse of ultrasound and neutrons at a cylinder of acetone in which the hydrogen atoms had been replaced by deuterium atoms caused bubbles to form, swell, and collapse. The heat and pressure at the center of the collapsing bubbles reportedly fused deuteriums together, liberating nuclear byproducts and excess energy.

The work raised the promise of limitless energy and spurred numerous early attempts to replicate it, all of which failed. Taleyarkhan moved to Purdue in 2004 and set about reproducing the original bubble fusion results. That winter and spring, according to the panel's report, Taleyarkhan's postdoctoral assistant Yiban Xu conducted bubble fusion experiments and wrote up the results, which were submitted to *Science*. The paper was rejected and later resubmitted to *Physical Review Letters*. *PRL* too rejected the paper; according to the panel's report, a reviewer commented that it was "unusual" that the experiment was done by one person "so that needed crosschecks and witnessing of results seem lacking."

In early 2005, Taleyarkhan asked Adam Butt, a master's degree candidate in his lab, to proofread the paper and check some of its numbers. After Butt did so, the panel says his name was added as an author of the paper, which was then submitted to *Nuclear Engineering and Design* (*NED*) and quickly accepted. "In this context, it is plain that the intent was to create the appearance of a joint author who participated in the experimentation itself," the panel's report concludes. "This is research misconduct." The panel flagged Taleyarkhan for a second count of misconduct for a 2006 *PRL* paper in which Taleyarkhan and colleagues cited the *NED* paper as proof of independent confirmation of bubble fusion. Although the panel concluded that several other allegations did not constitute scientific misconduct, the report was still deeply critical of Taleyarkhan's behavior and in some cases his scientific procedures.



Bubble bursts. A new investigation finds that Rusi Taleyarkhan misrepresented work from his lab as independent confirmation of his prior results.

In an e-mail to *Science*, Taleyarkhan says that the new report "is flawed from various perspectives and incorporates factual errors," though he does not spell them out. He adds: "The current state of matters represents a major setback for university faculty members in general—this sort of selective victimization to meet political-funding priorities of a huge institution (with relatively incomparable resources vs the sole individual) could happen to any other faculty member."

Kenneth Suslick, a chemist at the University of Illinois, Urbana-Champaign, and a longtime critic of bubble fusion, calls the report "some kind of vindication." Suslick says he was disappointed the report didn't more squarely address questions of possible scientific fraud that have been raised about the research (*Science*, 17 March 2006, p. 1532). The report states that although such allegations were made to a previous panel investigating Taleyarkhan's work, they were not forwarded to be made part of the current panel's investigation—but it does not explain why. The current report also did not attempt to evaluate the original scientific results behind "bubble fusion."

The latest panel was set up in March 2007 following complaints to the Inspector General of the Office of Naval Research (ONR), which helped fund some of Taleyarkhan's experiments. The panel was chaired by Purdue biochemist Mark Hermodson, and four of its six members came from outside Purdue University. Although the current panel submitted its report to ONR in April, it was formally accepted and made public only on 18 July.

Taleyarkhan's lawyer, John Lewis of Lewis and Wilkins LLP in Indianapolis, says Taleyarkhan plans to appeal the report's findings. However, he adds that he is "not optimistic" the appeal will succeed, given that it will be conducted by the university. Purdue spokesperson Joseph Bennett says that Purdue officials will not comment on the report until after any appeal is complete next month. The ONR letter states that the funding agency will keep the case open until Purdue takes corrective action to prevent similar occurrences in the future.

–ROBERT F. SERVICE

CREDIT: MICHAEL HEINZ/*JOURNAL & COURIER*/AP PHOTO

SCIENCE IN JAPAN

Paper Retraction Puts Focus on Informed Consent Rules

Stung by a front-page newspaper exposé of an alleged lapse in research ethics, officials at the University of Tokyo's Institute of Medical Science are planning to overhaul efforts to educate researchers on ethics rules and tighten internal review and compliance procedures. At the same time, scientific journals are getting stricter about documenting the fact that human studies have undergone ethics review.

"What we learned is that periodic educational efforts to raise everyone's awareness of clinical research ethics guidelines were insufficient," says Motoharu Seiki, the institute's dean.

The incident centers on a group led by Arinobu Tojo, who works on molecular therapies for leukemia. The group retracted a paper on acute myeloid leukemia by Seiichiro Kobayashi *et al*. that was published online on 21 May and in the 1 July issue of *Haematologica*. An investigation determined that a statement the authors made to the journal that the study had been approved by an institutional review board (IRB) was erroneous (*Science*, 18 July, p. 324).

Cold case. Research on archived human tissue, shown in this stock photo, has created an ethical quandary at the University of Tokyo's Institute of Medical Science and elsewhere.

Seiki says the group retracted the paper on its own initiative and that, to the best of their knowledge, there is no problem with the data, and patients were not put at risk. He says Tojo may have believed that approval of another aspect of his research covered this study as well. (No one has answered Tojo's office phone, and he did not immediately reply to an e-mail from *Science*.)

An outside investigative panel is now looking at other papers from the same lab. But Seiki says a key issue is that the retracted study relied on blood and bone marrow samples collected from six acute leukemia patients before the Ministry of Health issued its first guidelines for informed consent and IRBs in mid-2003. The guidelines allow the use of samples collected without informed consent only if approved by a review committee, but it doesn't clearly state that this requirement applies to old tissue samples or give any guidance on the use of such samples. Seiki says the institute needs to make researchers aware that use of these samples also requires IRB approval.

A whistleblower first alerted the institute's research compliance office of the Tojo group's alleged infractions. Seiki says the office discussed the issue with Tojo but didn't take the matter any further. The whistleblower later tipped off the *Asahi Shimbun*, a prominent daily newspaper, which contacted the institute, prompting the formal investigation. Seiki concedes the need "to put in place a system under which such mistakes are avoided."

Mario Cazzola, editor-in-chief of *Haematologica* and a hematologist at the University of Pavia in Italy, says this is the first time a paper has been retracted from the journal for a false or erroneous statement about ethical review. Editors at several other publications also think it is a first.

Journals appear to be paying more attention, Robert Dellavalle, a dermatologist at the University of Colorado, Denver, and colleagues wrote in an article in the February 2008 issue of *Clinical and Investigative Medicine*. They reported that 83% of 101 English-language medical journals surveyed require ethics committee approval of human research in their instructions to authors, up from 47% of 102 journals surveyed in 1995. And among those, 85% ask for a statement from authors attesting to such approval.

Journal editors' opinions are mixed on the effectiveness of requiring statements from authors, as opposed to the IRBs themselves. In an e-mail to *Science*, Margaret Winker, president of the World Association of Medical Editors and deputy editor of the *Journal of the American Medical Association*, said that in a recent forum discussion some members felt that a statement from the IRB is needed to guard against false disclosures. But Dellavalle, on the other hand, believes requiring authors to state that they have complied with ethical reviews is already filtering out improperly conducted studies.

The *Journal of the American Academy of Dermatology* has begun requiring a copy of the IRB approval letter. *Haematologica* now asks authors to include the name and e-mail address of the approving IRB or ethics committee, the date of approval, and the case identification number; it may decide to require a copy of the IRB's letter as well once a paper has been provisionally accepted. And *Science* is also discussing requiring the IRB approval letter, according to Katrina Kelner, deputy editor for life sciences. *Science* now requires a statement of compliance with informed consent and IRB approval for human studies, although it is not published.

Dellavalle thinks journals should require and publish a description of the approved experimental protocols. This would help keep researchers from getting approval for one experiment and doing another—and would protect human subjects and keep scientists honest. The checklist for papers on research involving human subjects is bound to get a bit longer.

–DENNIS NORMILE

CREDITS: D. NORMILE/*SCIENCE*; (INSET) HUMAN TISSUE RESOURCE NETWORK, DEPT. OF PATHOLOGY, THE OHIO STATE UNIVERSITY (WWW.PATHOLOGY.OSU.EDU/HTN)

SCIENTIFIC MEETINGS

Europe's Science Gathering Draws Crowds and Long-Term Funds

BARCELONA—Judging from the hallway talk, Europe's biennial science festival was a boon for this city's notorious pickpockets; tales abounded about participants who, after a stroll along the fabled Ramblas, spent the afternoon at a police station instead of debating biofuels or brain science. But that was about the only blemish on the EuroScience Open Forum (ESOF) 2008.

Online
sciencemag.org
Read much more about ESOF 2008 at Findings, *Science*'s news blog.

This year saw twice as many participants as attended the 2006 edition in Munich—some 4500 from 66 countries, including about 400 journalists—more sessions, and a greater variety of big names. Hatched only 4 years ago in Stockholm (*Science*, 3 September 2004, p. 1387), ESOF is fast becoming a key meeting point for scientists, policymakers, and reporters from around the continent; the meeting has come to embody the integration of dozens of national research cultures into something more European, says Norbert Kroó, vice-president of the Hungarian Academy of Sciences.

ESOF's format was inspired by the annual meeting of AAAS. ("A smaller AAAS meeting with better food," one participant quipped.) Both have a very broad scientific program, covering everything from nanotechnology to cosmology, and many public outreach events. ESOF's many policy sessions dealt with distinctly European trends and anxieties, however, such as international mobility or difficulties turning research into economic growth. At a session on innovation, for example, a speaker advocated the "dedemocratization" of R&D by betting more money on fewer groups, because Europe's tradition of spreading funding across countries isn't working.

ESOF is held in a different city every other year and, like the Olympics, is largely organized by a local committee and people they recruit from across Europe. The inevitable result has been the occasional reinvention of wheels, says Enric Banda, co-chair of the Barcelona committee. To address that problem, five private foundations—two each from Italy and Germany, and one from Sweden—announced on Monday that they have formed a "Supporters Club" that will pony up €1.6 million over the next 4 years to set up an ESOF secretariat. Right now, Euroscience, ESOF's parent organization, has only one permanent staff member.

The secretariat will take care of fundraising at the European level and serve as an institutional memory. But ESOF wants to retain the strong local involvement because it generates much more enthusiasm than a central organization, says Swedish physician Carl Johan Sundberg, who dreamed up the concept 10 years ago.

Making cities vie to host ESOF is also a way to improve future meetings. The northern Italian city of Torino, for instance, won the 2010 edition in part because it promised a very ambitious Web presence. Torino computer scientist Angelo Raffaele Meo has taken up the challenge of webcasting every session live, and remote viewers will be able to interact, for instance, by e-mailing questions for speakers.

Helga Nowotny, the Austrian chair of the 2010 program committee, said she'd also like to see more participants from Eastern Europe and from private businesses. Sundberg, for his part, says he'll strive for more audience participation. "Too often, it's still a series of talking heads," he says.

Dublin and Vienna have thrown their hats in the ring for ESOF 2012 and were lobbying heavily in Barcelona. Other cities that want to cultivate a brainier image have until 1 October to bid. **–MARTIN ENSERINK**

With reporting by John Travis.

Clear sailing. Outreach events such as this balloon-launched paper ship are a hallmark of ESOF, which has secured new funding for its future meetings.

CREDIT: ESOF 2008

Fighting for Peace

Archaeologists are urging their colleagues to "resist any attempts by the military and governments to be co-opted in any planned military operation" in Iran, a stance that some academics say is potentially disastrous.

Last week, the World Archaeological Congress (WAC) in Dublin passed a resolution warning that a conflict in Iran would have "catastrophic consequences for millions of people and will seriously endanger the cultural heritage of Iran and the Middle East in general." But the prohibition on providing governments with advice or expertise is "misguided and naïve," says Lawrence Rothfield, a cultural policy professor at the University of Chicago in Illinois.

Rothfield and others note that input from U.S. experts prior to the Iraq war in 2003 reduced the damage done to that country's rich archaeological past. Acknowledging the rift, WAC President Claire Smith said that the resolution was passed by the group's assembly but is not a formal position of the organization.

–ANDREW LAWLER

Ruling Protects Wolves

A U.S. federal judge has put the gray wolves of the northern Rocky Mountains back on the list of endangered species. Last week, U.S. District Judge Donald Molloy in Missoula, Montana, granted a preliminary injunction that restores federal protection to wolves in Idaho, Montana, and Wyoming and scuttles the three states' plans to allow hunting this fall.

In February, the U.S. Fish and Wildlife Service removed the wolves from the endangered list, saying that the estimated 2000 canids represented a conservation success story (*Science*, 15 February, p. 890). But 12 conservation organizations contested the decision, arguing that the government had not met its own criteria for success, in particular, achieving a genetically mixed population. In his 40-page decision, Molloy agreed that "genetic exchange has not taken place" and that allowing wolves to be hunted or killed if they attacked livestock would likely "eliminate any chance" for such exchange to occur. Molloy will eventually decide if the injunction should be permanent.

In the 5 months since the delisting, 110 wolves in the region have been killed due to state laws that permit citizens to shoot the canids as predators or if they "worry" livestock, says Louisa Willcox, a spokesperson for the Natural Resources Defense Council, a plaintiff on the suit.

–VIRGINIA MORELL

WENCHUAN EARTHQUAKE

Lessons of Disasters Past Could Guide Sichuan's Revival

BEIJING—Two months after an earthquake devastated a mountainous swath of Sichuan Province, urban planning experts met* here to take stock of how past disaster responses could guide a massive reconstruction effort about to get under way. One tragic lesson confirmed by structural engineers is the shoddiness of many schools and other buildings that collapsed—and the countless deaths that could have been avoided.

"This disaster creates an opportunity to rebuild a more resilient community," says Steven French, an urban planning professor at the Georgia Institute of Technology in Atlanta. As John Holmes, U.N. under-secretary-general for humanitarian affairs, puts it, "now is the moment to appraise, with sober wisdom, how to avoid the mistakes of history." Questions include where to rebuild communities and whether building codes must be strengthened.

The 12 May Wenchuan earthquake killed at least 70,000 people and left nearly 5 million homeless. China mobilized an army of soldiers and volunteers to get displaced people into shelters and set up makeshift schools. Last month, the State Council issued a decree ensuring scientific input into the reconstruction of a fractured area encompassing 20,000 square kilometers. "In very few disasters have I seen the consistency and rapidity of China's response," says Ede Ijjasz, a sustainable development expert in the World Bank's Beijing office.

But the harsh truth is that many building collapses could have been avoided. Engineers have concluded that scores of structures had poor ductility—the ability to sway with shaking or retain integrity long enough for people to escape. Reinforcement bars (rebars) in concrete beams and columns provide ductility. Observers have decried obvious cases of negligence, such as missing or grossly deficient rebars.

There were more subtle—but equally fatal—defects as well. A team from the Ministry of Education's Key Laboratory of Building Safety and Efficiency found that many collapsed structures lacked transverse rebars. "From the reconnaissance of the earthquake damage, it is clear that most of the damaged concrete building columns and beam to column joints did not have proper transverse reinforcement," states a report by lab director Xiao Yan, a civil engineer at the University of Southern California in Los Angeles and Hunan University in Changsha. "Transverse reinforcement is particularly important to confine the concrete." And in many instances, floors were not tied to support walls, allowing buildings to "split apart and collapse," says William Holmes of Rutherford & Chekene Consulting Engineers in San Francisco.

As experts dissect the damage, efforts to shore up buildings are under way. For example, a $58 million effort to reinforce columns and repair masonry is progressing fast at Southwest University of Science and Technology (SWUST) in Mianyang, which lost three students to the earthquake (*Science*, 30 May, p. 1145). Staff members are pitching in—the university canceled their summer vacations—and the campus is expected to reopen at the end of August, says SWUST mining professor Zhang Zhigui.

A bigger challenge is to rebuild villages wiped out by landslides and towns such as Beichuan damaged so extensively that they must be reconstituted from scratch. In hard-hit areas, "recovery is complicated, messy, ugly, and painful. It's an insane environment for professional planners," says Robert Olshansky, a disaster expert at the University of Illinois, Urbana-Champaign. Over the past several weeks, geophysicists have been revising active maps designating areas that are at risk of intense shaking in future quakes or are prone to landslides.

The Chinese government is now plotting out the details of Sichuan's reconstruction. One basic necessity is to move freely in the damaged region. Last week, Sichuan authorities told urban planners that it will cost $10.6 billion to revamp the province's transportation network. This will be one component of a reconstruction plan that China's National Development and Reform Commission says will be ready by September.

"The government's role should be to send lots of money and stay out of the way," says Olshansky. He points to the "remarkable" comeback of Yogyakarta, Indonesia, ravaged by an earthquake in May 2006. "Housing collectives in each village were given money," he says, and in just 2 years, "virtually all seriously damaged homes have been rebuilt." One experience that Chinese planners would not wish to repeat is that of Orléansville, Algeria, which was reduced to rubble in a 1954 earthquake. The replacement city—Al Asnam—was poorly built, says Ijjasz, and it too was destroyed in a 1980 quake.

Economic and emotional recovery in Sichuan could take years, experts say. "It takes a tremendously long time to recover from these kinds of events," says Lawrence Vale, an urban planning professor at the Massachusetts Institute of Technology in Cambridge. Although infrastructure in Kobe, Japan, was repaired within 2 years after a 1995 earthquake, many small businesses and major industries have not bounced back, says Haruo Hayashi, a disaster-management specialist at Kyoto University. "In Kobe, 20% of people say they feel they are still victims," he says.

One question preying on the minds of victims is where they will live when they move out of temporary shelters. Experts advise Chinese authorities to take such decisions with utmost care. "The tension at the core of recovery is speed versus deliberation," says Olshansky. Another conflict, he says, "is between what planners will be promoting and the plan in the minds of inhabitants: the predisaster city." Whatever rises from the rubble in Sichuan, it had better be structurally sound. **–RICHARD STONE**

Major surgery. Reconstruction at Southwest University of Science and Technology is in high gear; the campus should reopen next month.

CREDIT: COURTESY OF STEVEN PLUMMER

*China Planning Network City Resilience Roundtable: Rebuilding and Restoration After the Sichuan Earthquake, 16 July.

Hot topic. CDC is mapping areas that are most vulnerable to heat waves.

CREDIT: SCOTT HOUSTON/SYGMA/CORBIS

U.S. ENVIRONMENTAL POLICY

EPA Calls for More Studies on Health Risks of Climate Change

Long-term changes in climate can affect human health in myriad ways. Last week, a new report by the U.S. government's climate change science program outlined a research road map to help Americans adapt to a warmer climate.

"The bottom line is that there are very real health risks associated with climate change," says Centers for Disease Control and Prevention (CDC) epidemiologist Howard Frumkin, a reviewer of the report, which was written by a team of government and independent scientists under the direction of the Environmental Protection Agency (EPA). The report cites an expected increase in the range of vectors that carry Lyme disease and West Nile virus and a greater risk of diarrheal diseases following more frequent flooding. Experts in the field acknowledge, however, that the science cannot yet help local officials anticipate specific public health crises. "A city health director says to us, 'What's the infectious disease I need to prepare for given the changing climate?' That's a question we can't answer right now," says Frumkin.

The report calls for health-surveillance systems to include "climate sensitive pathogens and vectors." It recommends more studies to model the health impacts of climate-related events such as wildfires or floods, as well as regional climate models to provide scientists with higher resolution data regarding local climate forecasts. Most current studies of public health issues don't include a climate component, it notes.

The report doesn't address whether the federal government is spending enough on climate-related "human dimensions." But last year, the National Research Council estimated the amount at $30 million, a level it said was woefully inadequate. A House spending panel has proposed giving CDC an additional $7.5 million next year "to prepare and adapt to the potential health effects of climate change."

CDC now spends less than $1 million on climate-related programs, including a mapping project combining temperature, census, and public health data to identify the areas within cities where residents are most vulnerable to heat waves. CDC's Frumkin says several important studies remain on the drawing board because of insufficient funding. And the agency's new $27-million-per-year national effort to track health changes related to environmental factors such as allergens excludes waterborne vectors and other factors specifically linked to possible warming.

The report seems to have escaped the strong political headwinds that previous official efforts on the topic have encountered during the Bush Administration. Last year, the White House censored written congressional testimony by CDC Director Julie Gerberding that made many of the same points contained in the new EPA report (*Science*, 2 November 2007, p. 726). Former EPA official Jason Burnett said earlier this month that Vice President Dick Cheney's office directed the excisions because he didn't want her testimony to affect EPA's pending rulemaking, ordered by the Supreme Court, on whether greenhouse gases should be regulated due to their effects on human welfare. This month, the agency decided to punt the issue to the next Administration (*Science*, 18 July, p. 324).

While that storm was raging, however, drafts of the interagency report were circulating freely on a federal Web site. Several authors, including epidemiologist and health consultant Kristie Ebi, say they experienced no political meddling while preparing the document. **–ELI KINTISCH**

SCIENCESCOPE

Chips Ahoy

Computer scientists at Radboud University in Nijmegen, the Netherlands, can tell the world how they cracked a widely used security chip, a Dutch court has ruled. NXP, a Philips subsidiary, had asked the court to stop the academics from talking at an October conference in Spain and publishing their findings in the meeting's proceedings (*Science*, 11 July, p. 189). But the court said that exposing a flawed security system serves society's interest and is an acceptable form of expression. In a statement, NXP says that clients "may want to urgently review their [security] systems." The company is considering an appeal. **–MARTIN ENSERINK**

Hello, Fellows

The U.S. Food and Drug Administration (FDA) is hoping a new fellowship program will entice talented scientists and physicians to join the agency. Participants will spend 2 years learning about FDA's regulatory practices in drugs, devices, and food, as well as legal and policy issues. The agency plans to accept between 30 and 40 fellows this fall and grow the program if it's successful (www.fda.gov/commissionersfellowships). The program comes during a hiring bonanza aimed at adding 1300 employees by October.

–JENNIFER COUZIN

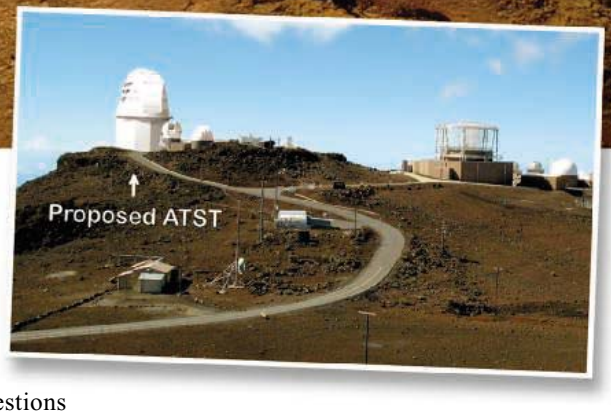
Monkey See, Monkey (No) Do

Leading researchers who study microbicides to prevent transmission of the AIDS virus are calling for more studies in monkeys (see p. 532), and—until last week—they had high hopes that the Bill and Melinda Gates Foundation would provide the funding for the expensive experiments. Their optimism was well-grounded: In February 2007, the foundation began courting four prominent groups of researchers to work together to answer fundamental questions about the safety and efficacy of several different topical agents that can be inserted into the vagina or rectum to derail the AIDS virus.

The researchers, who wrote proposals specific to the foundation's wishes, were elated because such studies are typically difficult to fund through the U.S. National Institutes of Health "unless you have preliminary data on each drug," says Ronald Veazey of the Tulane National Primate Research Center in Covington, Louisiana. But Veazey and other investigators received a curt rejection note from the Gates Foundation on 18 July indicating that the foundation had based its decision on "input from reviewers and internal decisions on foundation strategy for funding." **–JON COHEN**

From Atop a Mountain, A Deeper Look at the Sun

The world's largest solar telescope could help answer long-standing questions about our nearest star. But with environmental opposition and budgetary challenges, will it get built?

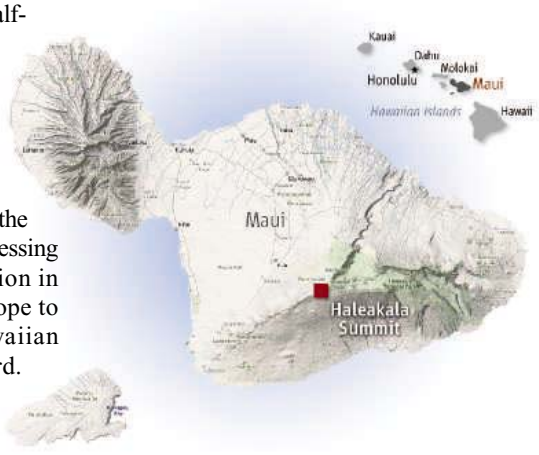


One morning in the ancient past, a Hawaiian legend says, the demigod Maui—after whom the island is named—stood atop the Haleakala shield volcano and lassoed the rising sun. He made the Sun promise to slow its journey, giving Maui's mother longer days to dry her tree-bark cloth.

Today, astronomers are hoping to use the Haleakala summit for a more scientific tryst with the sun. Supported by funding from the National Science Foundation (NSF), they plan to build the world's largest solar telescope there. The Advanced Technology Solar Telescope (ATST) is proposed to be 43 meters high with a 4-meter-wide mirror that will help researchers image the sun's surface in unprecedented detail. Researchers hope that observations from ATST will confirm theoretical models about the sun and answer fundamental questions such as the origins of sunspots and solar flares.

But as the project enters its final design stage, some fear that it may never see the light of day. Some local Hawaiians are campaigning to block construction of the observatory, arguing that ATST will ruin the grandeur of a mountaintop already cluttered with a half-dozen smaller telescopes. Meanwhile, a tough funding climate combined with a spiraling cost estimate poses a serious threat to the project.

Nonetheless, ATST's proponents within NSF and the National Solar Observatory (NSO) in Tucson, Arizona, the principal developer of the project, are pressing ahead with plans to begin construction in 2010. By the end of this year, they hope to reach an agreement with local Hawaiian groups to let the project move forward. And despite the uncertain budgetary picture, they believe ATST has a good chance of being funded, in part because the federal government has not invested in a ground-based solar observatory in decades. "This is transformational science for solar physics," says NSO's Jeremy Wagner, project manager for ATST. "This facility can answer fundamental questions about the sun that the solar community has been chomping at its bit to answer."



To stare at the sun

If it is built, ATST will be the first new ground-based telescope in a generation dedicated to studying the sun's vast, complex, ever-changing magnetic field. Solar astronomers hope ATST will reveal the contours of this field at a resolution of about 30 kilometers across the sun's surface—about five times the resolution of today's most advanced solar telescopes.

This level of detail could help astronomers unlock mysteries such as why sunspots occur where they do, why their numbers wax and wane on an 11-year cycle, and what suddenly causes coronal mass ejections—huge outpourings of hot solar gas that can destroy satellites, disrupt power grids, and jeopardize space flights.

The sun is also the most accessible laboratory for studying astrophysics, solar physicists say. "We have no way of studying other stars in detail," says Robert Rosner, a solar astronomer at the University of Chicago and a co–principal investigator on the ATST project. "With the sun, we have an opportunity to actually look at fundamental processes such as the generation of magnetic fields."

Efforts to build high-resolution solar telescopes run into the distorting effect of air turbulence on light collected by the telescope. ATST's designers counter this problem with a deformable mirror, mounted so that 1000 actuators flexing 1000 times per second can bend it to bounce off the light at the precise angles needed to correct for atmospheric distortion. "It's similar to how noise-cancellation headphones work," says Robert Hubbard, an engineer with NSO. An ensemble of 12 mirrors, including the deformable one, will ultimately guide the column of light into a charge-coupled device camera. Because magnetic fields polarize light in specific ways depending on their strength and direction, scientists will be able to map the magnetic field of the solar area being imaged.

By the time NSF officials picked the Haleakala site as their top choice out of six finalists, Haleakala's long sunny days, clear, tranquil skies, and dust-free environment had

For the view. Some residents of Maui say adding another telescope will spoil the beauty of the Haleakala summit.

long made it a popular site for astronomers. A 7-hectare expanse on the summit, managed by the University of Hawaii, is home to one nighttime observatory and two privately funded solar observatories run by the university; a U.S. Air Force surveillance observatory; and a robotic telescope run by the Las Cumbres Observatory Global Telescope Network.

Taking the heat

Many native islanders, however, resent the presence of so many telescopes on the mountain and don't want to see another one built. Officials of the Haleakala National Park, which borders the site, have raised concerns that the project could harm the park's environment and spoil the view for park visitors. In a letter sent last year to the National Science Board (NSB), NSF's oversight body, the National Park Service complained that NSF was ignoring issues such as the threat to native plant and animal species on the mountaintop—including the Hawaiian dark-rumped petrel, an endangered bird that nests there. NSF officials say these concerns are being addressed in an ongoing impact assessment: For example, there is a plan to monitor the nests with video cameras to determine if construction might be disrupting the birds' nesting cycle.

Cultural impacts might prove to be a bigger problem. As a designated historical site, the Haleakala summit is protected under the National Historic Preservation Act (NHPA). In 2005, after NSF officials announced that Haleakala had been chosen for ATST, agency officials began holding public meetings with islanders to discuss ways of mitigating the environmental and cultural impact of the project—a process required by NHPA. The leaders of ATST were keen to start these consultations early, especially in light of vehement opposition to a proposed NASA telescope atop Mauna Kea on the Big Island.

Kiope Raymond, a professor of Hawaiian studies at the University of Hawaii's Maui Community College, was hired as an interpreter for one of the early meetings. "I had no idea at the time what the project was about," says Raymond. Over 3 nights, he listened as NSF officials made presentations about the project and took questions from local citizens. "I was incognito to the NSF people—they thought I was on their side," he says. "And I heard them making remarks to the effect that the project was a done deal and that the public meetings were simply a formality. They were simply going through the motions."

Angered, Raymond joined a growing public campaign against the project. Last year, he and a handful of other citizens founded Kilakila o Haleakala (Majestic is Haleakala), an organization dedicated to blocking further development on the mountaintop. He argues that besides taking away some of the summit's awe-inspiring natural beauty, this will inevitably damage the "spiritual connection" that native Hawaiians feel with the place.

Not all opponents of the project are that uncompromising. Some community leaders have put forth proposals for mitigation such as NSF funding for a center for traditional Hawaiian navigation and astronomy. A proposal from the Maui Community College asks the federal government to provide funding for improving science and math education on the island.

"[NSF officials said] the project was a done deal. … They were simply going through the motions."

—KIOPE RAYMOND, PRESIDENT OF KILAKILA O HALEAKALA

"All that we are required to do … is show that we went through the process of considering environmental and cultural impact."

—STEPHEN KEIL, NSO DIRECTOR

"We have also discussed measures like limiting the lifetime of the telescope to a certain number of years and steam-cleaning all vehicles that go up to the construction site to minimize harm to its sacredness," says NSF's Craig Foltz, program officer for the project. He says efforts are under way to negotiate an agreement with different citizens groups before the end of the year.

Even if no agreement is reached, the project could still go forward, says NSO Director Stephen Keil. "All that we are required to do under NHPA is show that we went through the process of considering environmental and cultural impact," he says. Officials expect a final environmental impact statement to be ready by the end of the year.

However, opponents say proving that the process was duly followed may itself be tricky, leaving the door open to legal challenges like the one that scuttled the NASA project on Mauna Kea. Indeed, the Advisory Council on Historic Preservation in Washington, D.C., sent a letter to NSF last week asking the agency to justify why it did not wish to consider alternative sites for the telescope.

The fire next time?

Meanwhile, even though NSB last year added ATST to NSF's queue of future initiatives, funding for the project is by no means assured. NSF has asked Congress for $2.5 million in fiscal year 2009 to complete the final design of the facility; it must now pass a final review by the NSF director and NSB before the agency asks Congress for construction money for the project. If all goes smoothly, Keil and others expect NSF to ask for construction dollars in its 2010 request.

But the project will be competing for funds in a tight budget. ATST's architects have had to explain why its estimated cost, currently $253 million, has more than quadrupled since the telescope was first proposed 9 years ago. They pin the increase partly on spiraling steel and concrete costs.

ATST also faces competition from a dark-sky astronomy project: the $389 million Large-Aperture Synoptic Survey Telescope, currently undergoing a preliminary design review after which it will be considered by NSB. "NSF obviously has a limited budget and may not be able to fund multiple astronomy initiatives in parallel," says Foltz. "Solar astronomers could argue that the nighttime astronomy community is already getting the Atacama Large Millimeter Array [an international telescope project in Chile]; they have had Gemini [a twin-telescope observatory]—whereas this is the first ground-based solar telescope being funded by the federal government in 30 years."

That's exactly the argument Rosner makes in support of ATST. "In a world where there is an infinite amount of money, everybody gets to play, but when resources are constrained, disciplines need to take turns," he says. "We think it's the turn of the solar community now. We think ATST's time has come."

–YUDHIJIT BHATTACHARJEE

CREDITS (TOP TO BOTTOM): DANIEL GRANTHAM; DAVE DOOLING/NSO/AURA/NSF

U.S. RESEARCH EARMARKS

Building a Scientific Legacy on a Controversial Foundation

Most scientists deplore the practice. But the first wave of congressional earmarks for academic research created two centers that have stood the test of time

BOSTON—Age-related macular degeneration (AMD) is the leading cause of blindness among older adults in Western countries, and a major study has shown that antioxidants and zinc supplements may slow its progression. But last year, after poring through data from that study, the U.S. government–funded Age-Related Eye Disease Study (AREDS), Tufts University protein chemist Allen Taylor found preliminary evidence suggesting another way to stop this vision killer: Cut your intake of dietary carbohydrates. White bread and other foods with a high glycemic index, he argues, contribute to AMD by destroying proteins in the retina and lens and making them less able to remove damaged tissue.

Online
sciencemag.org
Podcast interview with the author of this article.

Taylor is a long way from winning over his colleagues. "He's identified an interesting association that needs to be replicated. But it could be only an epi [secondary] phenomenon," says Frederick Ferris, clinical director of the National Eye Institute, which funded AREDS and is about to launch a follow-up to test the value of other nutrients in slowing the progression of the disease.

Such skepticism is normal for human nutrition studies, which are notoriously hard to interpret. That's no problem for Taylor, head of the vision lab at the Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University. The center is the only U.S. research facility focused on the intersection of nutrition and older adults, and the financial support from the U.S. Department of Agriculture (USDA), combined with outside grants, helps Taylor and his colleagues pursue the type of long-term studies needed to unravel these complex relationships. It's also the product of the first major modern congressional earmark for basic science.

Earmarks—directives by Congress to fund specific projects that have not been requested by an agency nor vetted by congressional committees—have traditionally been used by legislators to build roads and other civic projects in their districts. They are often disparagingly called pork-barrel projects. Thirty years ago, Jean Mayer, then president of Tufts, hired a fledgling Washington, D.C., lobbying firm to persuade the U.S. Congress to fund academic research this way. The resulting $32 million appropriation allowed Tufts to build a commodious home for the nutrition center—a 14-story glass-and-stone structure on the university's health sciences campus in downtown Boston that includes a hospital and medical and dental schools.

Some 700 kilometers to the southwest, on the tree-lined campus of the Catholic University of America (CUA) in northeast Washington, D.C., Ian Pegg enjoys the fruits of another early example of a federal research earmark. In 1983 and 1984, his university—aided by the same firm, now Cassidy & Associates, that persuaded legislators to fund the Tufts center—received $14.2 million to erect a four-story science building. In addition to housing the physics department, previously scattered around campus, the building is home to the sprawling Vitreous State Laboratory (VSL) that Pegg directs.

Most scientists, and their professional organizations, look down their noses at earmarks. They see them as a threat to the merit-review process that most federal agencies use to fund basic science (*Science*, 16 December 1983, p. 1211). Many politicians agree. Senator John McCain of Arizona, the presumptive Republican nominee for president, has made an election promise to eliminate them, calling them a waste of scarce government resources.

But that criticism has done little to curb the flow of earmarked federal dollars to academic institutions. The CUA and Tufts earmarks, together with a similar one to fund a chemistry building at Columbia University, opened the floodgates. The amount for universities exceeded $2 billion in 2003, the last year that the *Chronicle of Higher Education* tallied them, and this year, AAAS (which publishes *Science*) calculated that the total for all types of research projects has reached $4.5 billion. "Once the genie was out of the bottle, nothing could put it back," says Robert Rosenzweig, a former president of the Association of American Universities (AAU), who in 1983 led the first academic protest against this new phenomenon. "In some ways, it's remarkable that universities had abstained for so long."

Pouring it on. Molten waste is poured from a platinum container as part of a process developed at Catholic University's Vitreous State Laboratory, built with a congressional earmark. (*Overleaf*) Lab director Ian Pegg holds a chunk of material after vitrification.

Earmarks tend to recede from the news once the money has been allocated. So *Science* wondered what had become of the two projects often credited with launching the earmark era in science. Was the research sustainable once the earmark was spent? If so, has it influenced the course of research?

There's no way to know if the Tufts and CUA earmarks were typical or whether the money would have been better spent if it had been awarded in the type of competitive process that most scientists prefer. But there's abundant evidence that both projects have evolved to occupy unique niches. The Tufts center has contributed to an understanding of nutrition and aging, and the CUA earmark has improved the U.S. capacity to immobilize the detritus of its nuclear weapons program.

"I'm not in favor of earmarks, but this one [at Tufts] was certainly a good investment," says Walter Willett, a Harvard University epidemiologist who has worked with Taylor. "A lot of what they are doing may not be sexy, but it's critically important."

"Ahead of its time"

The Jean Mayer nutrition center sits on the edge of the crowded, commercial Chinatown section of Boston. It contains office and lab space for 68 scientists and a total of 340 employees, as well as living, recreational, and eating accommodations for volunteers participating in clinical nutrition trials. The in-house facilities allow scientists to closely manage the diets of patients enrolled in dozens of studies every year. "We're a

CREDITS: GREG SCHALER

Noah's ark for diseases that affect the elderly," says Taylor, who joined the center when its building opened in 1982. "There's one scientist working on this and two working on that."

Mayer, a prominent nutrition scientist, hatched the idea for a federally funded center shortly after leaving Harvard to become president of Tufts in 1976. He had helped organize the 1969 White House Conference on Food, Nutrition, and Health and thought that nutrition could become a signature program for the university. USDA was already conducting nutrition research at its Beltsville, Maryland, facility, and Harvard and the Massachusetts Institute of Technology (MIT) in Cambridge had more prominent academic programs at the time. But Mayer believed that the government needed an academic-based center to study the relationship between aging and nutrition and that Tufts was the best place for it.

To promote the idea, Mayer hired Kenneth Schlossberg and Gerald Cassidy, who had just opened up shop as lobbyists after staffing a Senate select committee on nutrition that grew out of the White House meeting. Together, they won over the powerful Massachusetts congressional delegation and House Speaker Thomas "Tip" O'Neill, whose district included Tufts. After O'Neill squashed a last-minute attempt by the other Boston-area schools to make it a multicampus center, Congress ordered the agriculture secretary to establish both a comprehensive human nutrition research program that would expand the department's existing efforts and a facility at Tufts that would focus on adult nutrition. One year later, in 1978, legislators committed construction money and put the project under a new science and education division at USDA that included its Agricultural Research Service (ARS).

"[Tufts] would not have done it on their own," says center director Robert Russell, who was recruited in 1980 by the founding director, Hamish Munro, a protein chemist at MIT. "Neither would a large, hard-to-move bureaucracy like USDA. But I think they are proud of what we have done."

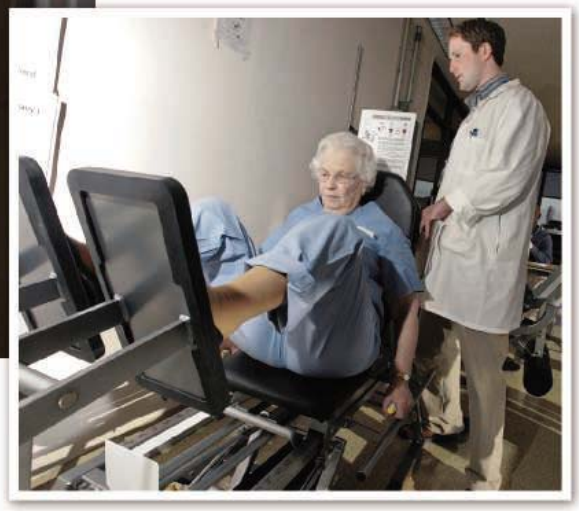
"It's an outstanding center," agrees Joseph Spence, former head of the human nutrition program and now area director at ARS, which provides $12.7 million a year for program activities. "We don't have clinical capabilities at Beltsville. By establishing a center at a medical school, we were able to combine agricultural production with human nutrition. And the emphasis on aging was ahead of its time."

The idea for a center was a no-brainer to Russell, a gastroenterologist then at the University of Maryland who was running a clinical nutrition program at a Veterans Administration (VA) hospital at the time. "[Munro] wanted to study the links between nutrition and age-related degeneration and the chronic diseases of aging," recalls Russell, who is leaving this month after 27 years at the center, the last seven as its director. "I was working with a malnourished VA population. And when I looked at the pending demographic changes, I realized that we would be entering uncharted territory."

Part of that growth involved defining the center's boundaries and scope. "USDA wasn't anxious for our people to apply for NIH [National Institutes of Health] grants because they wanted it to be a USDA program," says Russell. "So we had turf battles." Irwin Rosenberg, a gastroenterologist who succeeded Munro as director in 1986, also struggled to carve out a medical niche within the agriculture community. "I fought with USDA when they said, 'NIH does disease; you should do healthy people.' They were reluctant for us to use words like 'osteoporosis' or 'Alzheimer's.' But we eventually won those battles"—and access to NIH funding—says Rosenberg.

Outsiders concur that the first few years were rocky. "They got off to a slow start, but under Rosenberg's leadership things really picked up," says nutrition scientist Cutberto "Bert" Garza, a senior official at Boston College who has managed food and nutrition programs around the world. And Garza has a quick answer when asked how they succeeded: "They did it like any university, by recruiting better people."

The approach has translated into a steady stream of outside funding, including hard-to-get NIH grants. As of last year, for example, 18 researchers at the center had a total of 25 active R01 grants, the bread-and-butter NIH award for individual investigators. And their success rate for the past 3 years has been 28%, an enviable achieve-



Aging well. Robert Russell and Irwin Rosenberg flank a bust of the late Jean Mayer, who obtained an earmark for Tufts to build the USDA Human Nutrition Research Center on Aging that bears his name. (*Inset*) An 84-year-old volunteer exercises during a study.

ment given the stiff competition for NIH's flat budget. Overall, the center now receives almost half of its $22 million annual budget from other sources.

Still, the center couldn't do its job without USDA's annual contribution. "It's a commitment that's rare in nutrition research outside of a hospital," says Rosenberg, who stepped down as director in 2002 but maintains an active research program. "It gives us an institutional base for planning human nutrition research on a scale of a decade rather than just a few years."

Unfortunately for the center, that contribution has remained flat for the past several years even as the cost of research has risen. As a result, Tufts officials were forced to lay off six people last year, and Russell says the gloomy fiscal outlook is one reason—along with his age, 67—that he's stepping down as director: "The job is a lot more fun when the money's rolling in." Russell is joining NIH's Office of Dietary Supplements to work on strategic planning.

Nevertheless, the university hasn't lost faith in the center. Mary Teka, Tufts's vice president for university relations, says she spends "a fair amount of her time every year" shoring up support among the Massachusetts delegation for ARS's human nutrition program by disseminating the center's accomplishments. "This is a very high priority for the university, and we want to maintain its high quality," she says.

Clear solutions

Hidden in plain sight in the middle of the CUA campus, the VSL fills the warehouse-sized basement of a four-story building. The lab's most notable feature is a 10-cubic-meter tank, holding 2000 kilograms of molten glass maintained at 1150°C, that is a one-third-scale prototype of the melter system being built for the $12 billion Waste Treatment and Immobilization Plant (WTP) in Hanford, Washington. Its job is to help dispose of 200 million liters of radioactive and chemical waste now sitting precariously in 177 metal tanks in Hanford. Through their work on characterizing the properties and composition of glass, VSL scientists have a long and successful record of contributing to government projects aimed at immobilizing nuclear waste.

The university got into the nuclear cleanup business by accident. In 1967, the physics department won a competitive grant from the Department of Defense as a center of excellence in glass science to apply its work to potential military hardware such as night-vision sensors and high-powered lasers. The scientists—Theodore Litovitz and two of his students, Charles Montrose and Pedro Macedo—decided that the $600,000 award, a princely sum for those days, warranted a more formal name for their workplace.

The grant helped the group, renamed the Vitreous State Lab with Litovitz as director, become a pioneer in fiber optics. Working on ways to improve the refractive index of the glass fiber to enhance the flow of light along the cable, the scientists replaced the sodium in the glass with potassium and, then, cesium. But the ability to extract cesium from solution also turned out to have important applications in cleaning up the vast amounts of water used by commercial nuclear power plants, which produce isotopes of cesium that are radioactive. Further refinements of that ion-exchange technique were licensed to a spinoff company, Duratek, which went public in 1984 and last year became part of Energy Solutions, a nuclear fuel cycle company based in Salt Lake City, Utah.

Pegg says the university wouldn't have built a new home for the lab on its own. "We weren't at the top of its list of priorities, that's for sure," he admits. But CUA President William Byron decided to use the school's unique status as "The" Catholic University to seek help from Speaker O'Neill in addressing the lab's pressing need for more space to handle a growing workload. He hired Cassidy and Schlossberg, who also took on Columbia University as a client. In 1983, each school received a $5 million down payment—CUA got the rest the next year, and Columbia ended up with $20 million for a chemistry building—with the money carved out of the Department of Energy's science budget.

More than 20 years after he joined the lab, Pegg says colleagues still ask what attracted him to CUA. The answer, he says, is its unwavering focus on glass sciences. That's increasingly rare in a discipline now dominated by materials science departments, says the British-born Pegg, who was trained as a physical chemist at the University of Sheffield, U.K. And staying the course has paid off for him and the lab.

In the 1990s, Duratek joined with VSL to carry out the only completed pilot project to date of the technology chosen by the federal government to vitrify—immobilize in glass—such hazardous wastes. The lab is currently providing technical support for the company's role as a subcontractor for a piece

of the WTP project, the last stop for the waste before it is entombed in an underground repository in the Nevada desert.

The lab's extensive facilities for the characterization and testing of materials, including five glass melters, are unusual for an academic setting. But they are well-suited for the WTP project, which, after several delays, is now scheduled to be completed in 2019. "They're known for their scientific acumen, and the work they do for us is geared to solving specific problems," says Ivan Papp, a process engineering supervisor for Bechtel National Inc., the main contractor for WTP. In the past 7 years, for example, VSL scientists have tested some 500 glass formulations tailored to the different types of waste that must be processed.

Since moving into its new digs in 1987, the lab has employed as many as 110 people, on a budget of $13 million. That peak came in 2003, at the height of its work for Duratek on the Hanford project. The current staff of 67 includes 30 Ph.D.s, of whom five are tenured or tenure-track faculty members in the physics department. Although VSL scientists publish regularly, the lab's emphasis on contract work limits the scope of their research. "The scientific impact of their work is not so evident," says Rod Ewing, a mineralogist and materials science professor at the University of Michigan, Ann Arbor, and co-chair of a 1995 National Research Council workshop on the topic.

Ewing says the science of vitrification is more advanced in France and elsewhere in Europe because of the contrasting requirements for vitrifying and entombing their nuclear waste. "In the United States, the emphasis is on making a consistent product and relying on the container to last a long time, so the glass is less important," he says. "In contrast, the French are trying to understand the long-term behavior of the glass within a repository and the conditions that would affect its long-term durability."

Pegg doesn't dispute Ewing's assessment, but he thinks Ewing is comparing nuclear apples and oranges. "In France, they reprocess the waste, extracting the uranium and plutonium before converting it into glass," says Pegg. "The glass itself is the barrier. In the United States, the waste is mostly fuel assemblies, coming straight out of the reactor. So the repository has to be the barrier."

An uphill fight

Back at Tufts, Taylor and his colleagues are collecting evidence from animal and in vitro studies and combining it with data from population studies in other countries that have found a similar correlation between dietary carbohydrates and AMD. He suspects that excess carbohydrates or their derivatives modify retinal (and lens) proteins, rendering them less functional. They also modify the proteases (protein-degrading enzymes) that would otherwise recognize and eliminate the damaged proteins.

Paul Mitchell, a professor of ophthalmology at the University of Sydney, Australia, who leads a longitudinal eye study in Australia that has found a high glycemic food index to be an independent predictor of the risk of AMD, believes that dietary carbohydrates will turn out to be as important as antioxidants for the general population. (Among smokers, cigarettes are by far the leading cause of the disease.) And he credits Taylor for making an important contribution to understanding the importance of the dietary glycemic index, a topic that he says "is a real buzz area of medicine."

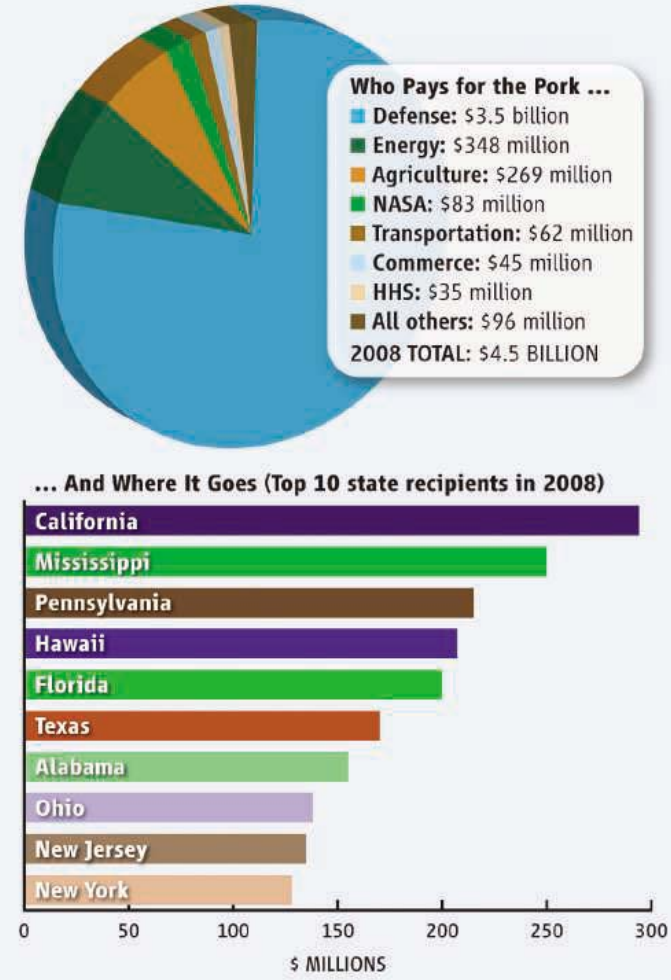
Because none of the researchers and administrators now at Tufts and Catholic universities played any role in obtaining the initial money for their school, none feels the need to justify it. For them, the enabling earmark is a piece of historical trivia. Not so for former president of AAU Rosenzweig. Although he's long since retired from the science policy arena, the subject can still get his blood boiling.

Rosenzweig came to AAU in 1983, when CUA and Columbia won their much-publicized earmarks. He mounted an immediate counterattack. "It sent a message," he recalls. "If Columbia could do it, then it was all right for everybody else." In October 1983, the AAU presidents passed a resolution in favor of the "processes based on the informed peer judgments of other scientists." The vote was unanimous, as Byron and Columbia's president, Michael Sovern, abstained.

Rosenzweig's prediction came to pass, and for better or worse, earmarks are now a firmly established feature on the federal science landscape. Whether individual projects have been successful isn't the issue, says Rosenzweig. "I never heard a university say that the money wasn't going to a worthy project," he says. "And I never challenged the work itself. What I said was, 'If you're spending government resources on something as important as research, then you should have a process that assures it is put to the best use.' Looking back, I feel justified in leading the fight, … for all the good it did me."

–JEFFREY MERVIS

Research Earmarks



A costly legacy. Congress approved $4.5 billion this year for research earmarks, continuing a 30-year upward trend that began with Tufts.

SOURCE: AAAS, 2008 APPROPRIATIONS

PLANETARY SCIENCE

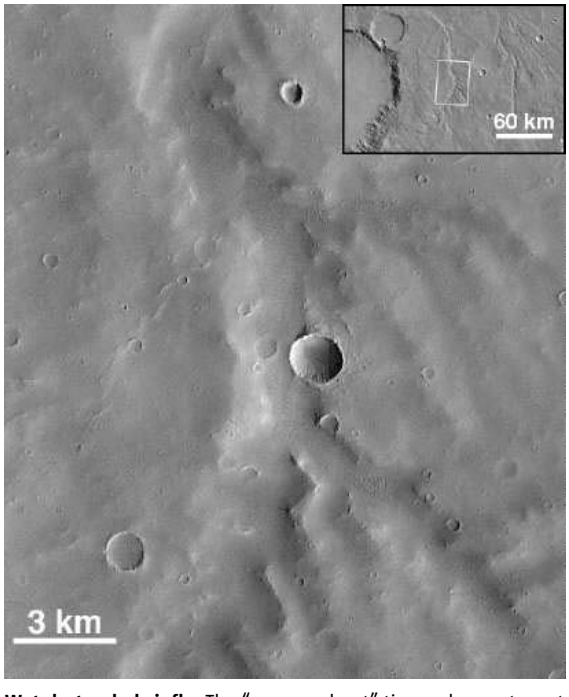
Water Everywhere on Early Mars But Only for a Geologic Moment?

Planetary scientists pursuing water and life on Mars must reconcile mounting evidence of a young planet awash in life-sustaining water with a growing realization that the martian surface was likely almost always dry

The early Mars of human imagination has swung from invitingly moist to brutally dry and back again more than once in the past half-century. Hopes for some sign of life that might have struggled into existence in that first billion years of martian history have likewise been alternately buoyed and dashed. In recent years, much of the news has been decidedly wet: a shallow, salty sea, rivers languidly flowing into crater lakes, and an exploding palette of spectral colors denoting water-altered minerals.

Most of these sorts of once-wet martian features are in the running to be the lone landing site for the next U.S. Mars rover, the $1.9 billion Mars Science Laboratory (MSL). Its mission: "follow the water" to understand whether the planet has ever been able to support microbial life.

But while the media were touting these striking examples of "sustained" liquid water on early Mars, less heralded evidence has been pointing to far less hospitable conditions. "I'm absolutely convinced there were periods of time when there was a very moist climate," says longtime Mars geologist Michael Carr of the U.S. Geological Survey in Menlo Park, California, but "I'm skeptical the wet conditions were persistent." In fact, says Mars fluvial geologist Robert Craddock of the National Air and Space Museum (NASM) in Washington, D.C., "the thinking has shifted to maybe punctuated, short-lived" episodes of warm and wet conditions. The rest of the time—maybe 999 years out of 1000—the surface of Mars would have been cold and drier than any desert on Earth and less hospitable to life.



Wet, but only briefly. The "warm and wet" times when water cut this valley network could have lasted mere centuries.

Dry, with wet moments

The vision of a wet early Mars got a big boost in 2004 when the Opportunity rover spied fossil sand ripples now exposed on the Meridiani Planum of Mars. Only water flowing on the surface—not groundwater—could have formed ripples with their particular shapes. So was born the "shallow, salty sea" of early Mars, which has been drying up ever since. Rover team members have been increasingly emphasizing that almost all of the salty sediments of the Opportunity site were laid down as windblown dunes that were later altered by briny, acidic groundwater (*Science*, 5 January 2007, p. 37). That groundwater may have oozed to the surface to puddle between dunes once in a great while, allowing those water-formed ripples to form, but even then it may have been too briny for even the most salt-tolerant life known on Earth (sciencenow.sciencemag.org/cgi/content/full/2008/529/1).

Another way to have sustained, continual water flow on the surface of Mars has also lost ground of late. Imaging by the Viking orbiters in the late 1970s revealed so-called valley networks—branching channels up to several hundred meters deep—that formed during the Noachian geologic era of Mars, roughly the planet's first billion years. Many planetary geologists thought the channels were carved by water seeping up from underground. Springs emerging at the head of each valley would have weakened the rock of the valley head wall, causing it to collapse into an amphitheater shape, then the spring water would have slowly eroded the debris away. Such seepage, or "sapping," was supposed to have formed some similar amphitheater-headed canyons on Earth. But a recent study published in *Science* (23 May, p. 1067) presented strong evidence that one classic sapping valley, Box Canyon in Idaho, resulted from one or more catastrophic floods.

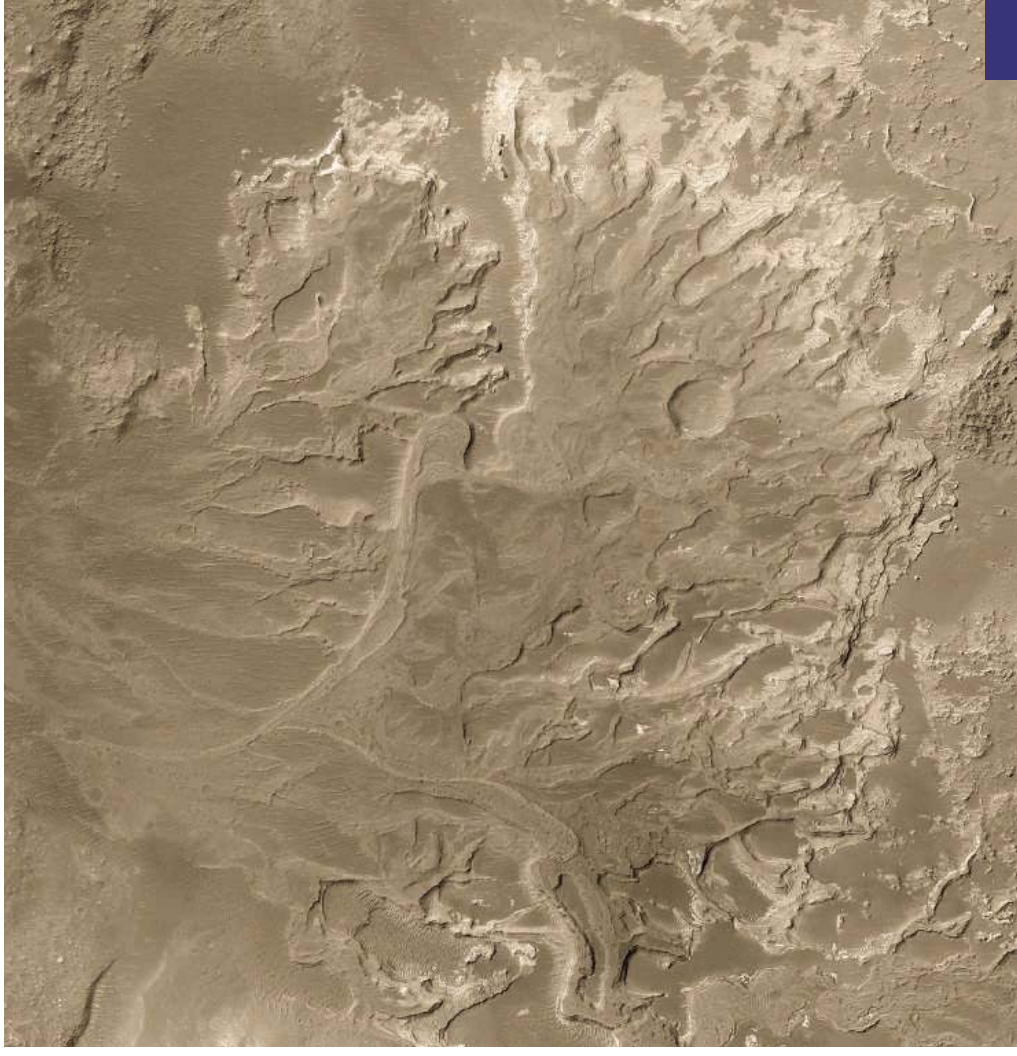
Now most experts think the treelike, twig-to-branch-to-trunk valley patterns on early Mars came about the same way most such patterns do on Earth: as drainage systems of rain-fed streams and rivers. But how could even the scarce rain of, say, the Nevada desert—going on for the better part of a billion years—leave so much of early Mars still standing? "Maybe Mars wasn't even like Nevada," says planetary geologist Ross Irwin of NASM. Maybe it was only something like Nevada once in a great while.

Fluvial geomorphologists Sanjoy Som and David Montgomery, both of the University of Washington (UW), Seattle, presented some of the latest evidence for such ultrarare rain at the Astrobiology Science Conference in Santa Clara, California, this past April. Using orbital imaging and altimetry, they compared signs of how water had flowed through 10 of the largest martian valley networks with flow-related characteristics of terrestrial drainage systems. For example, prolonged flows on Earth tend to widen channels and flatten their slopes downstream. The researchers concluded that even in the Noachian, Mars was much as it is today—cold and dry—with only rare episodic gushes during brief warm and wet intervals.

Early Mars was like the Atacama Desert of the high Andes, only more extreme, says planetary scientist Tomasz Stepinski of the Lunar and Planetary Institute in Houston, Texas. Stepinski says the UW analysis supports conclusions he and others had reached earlier by mathematical analyses of valley network patterns: "If there was precipitation, it was probably in the form of bursts. Mars didn't have the time to develop the intricate [drainage] patterns seen on Earth." Instead, he says, martian erosion works the same way it does on the Atacama: by prolonged dryness punctuated by an extraordinary gully washer of a storm.

On Mars, "it probably wouldn't have taken millions of years" of rainfall to carve the val-

CREDIT: NASA-JPL



How persistent? The sediment-laden flows that formed Eberswalde delta could have been short-lived.

CREDIT: NASA-JPL

ley networks, says Craddock. "Our best estimate is that valley networks were only active hundreds or at most thousands of years. It may have been punctuated events scattered over a long time of martian history."

Paradoxical puddles

While valley networks were pointing toward an almost-always-dry early Mars, planetary scientists were also finding signs that early Mars was wet—so wet that water drained into crater lakes. Press releases heralded this evidence for "pervasive and long-lasting" wetness that had "the potential to support life." But some researchers say that those lakes—which include three or four out of seven sites in the final running for the MSL rover landing—could have filled and dried up again within a geological moment.

The latest evidence for a persistent lake on early Mars comes from 45-kilometer Jezero crater, planetary scientists Samuel Schon, Caleb Fassett, and James Head of Brown University reported at the Lunar and Planetary Science Conference in Houston last March. Examining the latest high-resolution images from Mars Reconnaissance Orbiter (MRO), they point out features—such as distinctive cross-bedding of sediment layers—that form only when river channels meander across a delta building out into a lake. And delta channels can meander only if the standing water the delta is growing into remains more or less steady at one level. That implies a "long-lived" lake in Jezero crater, the group says, much as proposed for 65-kilometer Eberswalde crater, a potential MSL target.

The problem is that no one can put a number on "long-lived." "There's almost no question these [crater] deposits are fluvial," says fluvial geomorphologist Douglas Jerolmack of the University of Pennsylvania, but "there's also a lot of evidence they could have been done very rapidly." In 2004, Jerolmack and colleagues used a computer model to estimate that Eberswalde's fan of sediment could have formed in several decades to centuries. Fluvial geomorphologists Montgomery and Craddock both agree that geologists could well be looking at lake deposits built up by one or more brief gushes down valley networks like the one that fed Jezero.

Watery colors

The latest watery news of early Mars came last week, when *Nature* published the most comprehensive spectral survey yet of Mars, setting off a media storm. The MRO spectrometer discovered the spectral signatures of clays—the product of prolonged water weathering of rock—at a couple of thousand sites. Mars was a warm, soggy, water-logged planet for hundreds of millions of years in its early history, news stories proclaimed.

The *Nature* paper by planetary scientist John Mustard of Brown and 35 MRO colleagues was a good bit more restrained than its media coverage. Although it pointed to the existence of clays, it never placed them definitively on the surface of the planet. Primitive life might have arisen in the subsurface, Mustard notes, ever so slowly feeding on chemicals from the rock. But nothing in the spectral data contradicts the valley network picture of a surface almost always dry.

If the "punctuated precipitation" model is correct, how did Mars generate such rare, geologically momentary episodes of rain? Some researchers credit cosmic collisions. In a 2002 *Science* paper, planetary scientist Teresa Segura of Northrop Grumman Space Technology in Redondo Beach, California, and colleagues proposed that large asteroids or comets striking the martian surface flung hot rock and rock vapor around the planet; the heat vaporized water and ice stored beneath the surface and in the polar caps, driving it into the atmosphere (*Science*, 6 December 2002, p. 1866).

Not everyone was convinced. "I had problems with the *Science* article," says Craddock, as did others. The atmospheric physics of Segura's model was incomplete, some noted, and the very large impacts they modeled would have come too early in Mars's history to explain the observed fluvial geology, among a number of criticisms.

Segura and colleagues listened and are back with a new and improved paper that is in press in the *Journal of Geophysical Research*. They added more atmospheric processes to their model and considered smaller, later arriving impacters. The more detailed model supports the conclusions of the earlier one: Impact-induced greenhouse conditions could have lasted for centuries, driving up to 18 meters of rainfall. The group calculates that rainfall due to Noachian impacts would have eroded away at least 50 meters of the planet's surface—roughly the amount of erosion that planetary scientists estimate actually occurred. The once-doubtful Craddock thinks that "what they've done now is the best solution we have. Now we need to test it a little."

–RICHARD A. KERR

How strong do you lean? Twin studies suggest that the intensity of a person's partisan attachment, and even whether that person votes, may be influenced by genes.

Voting: In Your Genes?

Behavior geneticists say almost every human behavior that can be reliably measured—from TV-watching to optimism—is significantly influenced by genes. Now they're extending their reach into the voting booth.

Numerous studies over the past 2 decades, the first led by psychologist Nicholas Martin at the Queensland Institute for Medical Research in Australia, have indicated that genes have a significant influence over whether you're "liberal" or "conservative" on various political and social issues. Some heritability estimates have been as high as 50%. That's roughly the heritability found for many personality traits such as "extraversion" or "agreeableness," and it implies that, in a given population, about half of the variation in a particular trait is attributable to genetic differences.

Now James Fowler, a political scientist at the University of California, San Diego, and grad student Christopher Dawes say they've produced fresh evidence that DNA also has a hand in the intensity of someone's partisan attachment and even in whether someone bothers to vote.

As they reported here, they did that by crunching data from twin registries and the government's long-running National Longitudinal Study of Adolescent Health (NLSAH). In one study, the researchers matched data on voting by 396 Los Angeles–area twins, including identical (who share 100% of their genes) and fraternal (who average 50% genetic overlap) twins, obtained from Los Angeles voter-turnout records. All twins were same-sex pairs to avoid confounding results with sex differences. The researchers corrected for environmental factors such as whether more of the identical than fraternal twins were living together, which might inflate their degree of similarity. The researchers concluded that the correlation for voting was much higher between pairs of identical (.71) than fraternal (.50) twins. From this they estimated the heritability of voting behavior—that is, whether people eligible to vote actually do so—at 53%, suggesting that at least half the individual variation can be traced to genetic influences. They found an even higher heritability—72%—when they replicated the study with data on 806 twins from NLSAH, they reported in the May issue of the *American Political Science Review.*

The San Diego researchers also argued for a biological twist to how strongly someone identifies with a given party. The group, led by grad student Jaime Settle, gave questionnaires to 353 pairs of same-sex twins in Twinsburg, Ohio, where twins from all over the world hold a summer gathering every year. The twins were asked to rank their partisan attachment on a seven-point scale. From that, the researchers report in an as-yet-unpublished paper, they calculated a heritability of 46% for party loyalty, independent of affiliation. Whereas "partisan direction" seems mainly influenced by social and demographic factors, the researchers conclude, "partisan intensity" is not.

Several groups are now trying to correlate personality data with DNA markers from studies such as NLSAH, which contains DNA as well as behavioral data from many subjects, in hope of identifying specific genes that feed into underlying traits, such as "desire for cooperation," that Fowler, for one, believes have been selected for throughout human evolution. Studies so far have focused on the same genes that are of interest in psychiatric genetics—in particular those involved with neurotransmitters such as dopamine and serotonin that are known to be important in regulating higher brain activities.

The heritability studies are "intriguing," says David Goldman, chief of the neurogenetics lab at the National Institute on Alcohol Abuse and Alcoholism in Bethesda, Maryland. But he is skeptical about attempts to translate findings from twin and family studies into molecular hypotheses. "In any questionnaire you'll find heritability," he says, "but you don't know what's being inherited." So "it's premature at best to attack a complex phenotype like political leanings at the molecular level."

Martin, who pioneered twin studies on such behaviors, applauds social scientists for plunging into biological and evolutionary issues. At least some "are starting to acknowledge that humans are genetically unique individuals and not just cloned pawns" of their environment. And that suggests that prophets and pundits, however prescient, are probably never going to get much better at predictions than they are now.

CREDIT: CARLOS BARRIA/REUTERS /LANDOV

The Sociable Brain

What was the selective benefit of a big brain? Anthropologists have long puzzled over what drove the astounding growth of the hominid brain, which, unlike that of any other primate, tripled in size over 2 million years.

Behaviors such as language and tool use have been postulated to drive the evolution of cognitive abilities hand-in-hand with brain size. One theory that's garnered great attention has been "the social brain" proposed by University of Oxford anthropologist Robin Dunbar, who suggests that human brain size was selected in particular for capabilities, such as the ability to sense what another person is feeling, that are useful in social groups. Dunbar has backed this theory with evidence that among primates, species that form larger social groups have larger neocortexes on average.

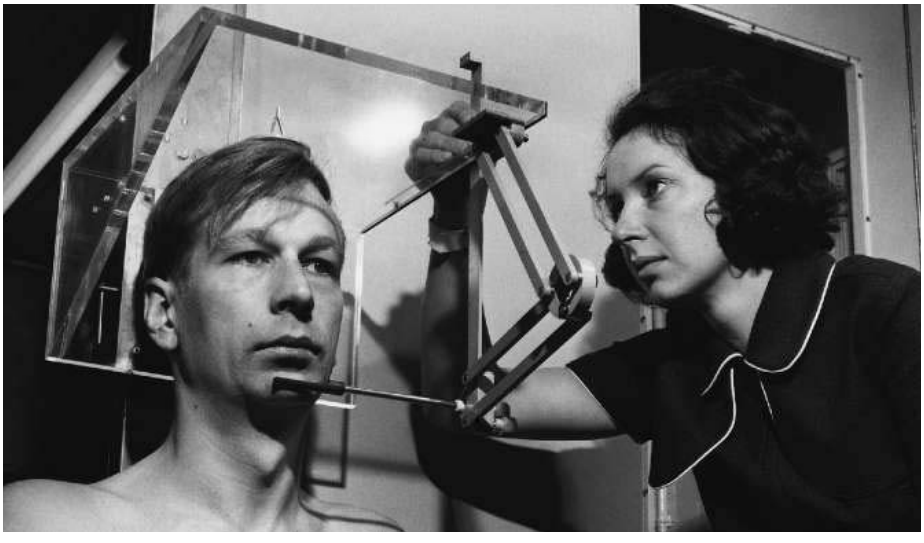
Timothy Bates, an evolutionary psychologist at the University of Edinburgh, U.K., decided to give Dunbar's theory a field test on a "complete social system": 200 young primates living in Baird House, a student college at the university. "If the social brain theory is correct, head size variation between humans should be reflected in individual differences in support and sympathy group size," he reasoned, so that people with bigger brains should have larger circles of friends.

Past studies have shown that head size is strongly correlated with brain size (about .60), and brain size has a low but significant correlation (about .30) with IQ. (The IQ–head size correlation is even lower at about .20.) Bates and his helpers gave the male and female students IQ tests and measured their heads to get rough estimates for brain size. He also gave them personality and "theory of mind" tests to probe how well they thought they could sense the feelings and motivations of others. Finally, the students were asked to assess their relationships to everyone else in the dorm, indicate how many friends each had, and rank closeness of friendships on a scale of one to three. Bates was able to crosscheck the data by asking students to indicate which of their peers were "well-liked."

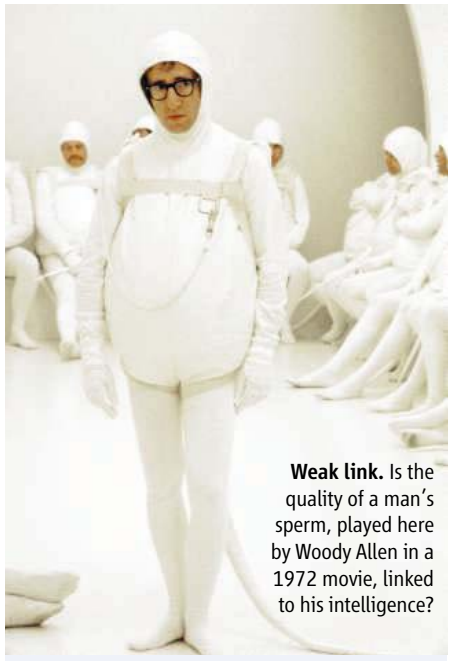
The result? Head size correlated modestly with IQ. But neither head size nor IQ correlated with the number of friendships, Bates reported at the meeting. "Most variance in social group size was explained not by cognition but by personality traits, especially extraversion," he says. This suggests that the human ability to form large groups may depend on selection not for advanced cognitive abilities but for "agreeable" personality traits, says Bates. The evidence in this experiment suggests that "the sole correlate of the unique size of the human brain appears to be intelligence."

Evolutionary psychologist Geoffrey Miller of the University of New Mexico in Albuquerque calls Bates's experiment "a creative and revealing new way to shed light on the social intelligence hypothesis"—even though it's "not a clear disproof." Miller himself thinks "our runaway brain evolution" must have been helped along by additional factors including sexual competition and technological innovation.

–CONSTANCE HOLDEN



Social intelligence? People with bigger brains don't seem to have more friends, as one theory would predict. Above, an RAF pilot being measured for a uniform in 1970.



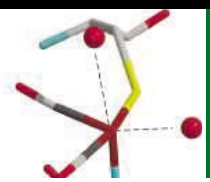
Weak link. Is the quality of a man's sperm, played here by Woody Allen in a 1972 movie, linked to his intelligence?

Do Good Sperm Predict a Good Brain?

Could high-quality brains and high-quality sperm travel together? Some scientists wonder if there is a "latent fitness factor" that would cause evolutionarily desirable traits, both physical and mental, to be correlated with one another.

Rosalind Arden, a psychology grad student at University College London, with evolutionary psychologist Geoffrey Miller of the University of New Mexico in Albuquerque and sociologist Linda Gottfredson of the University of Delaware, Newark, decided to test the question by looking for a correlation between IQ and sperm quality, a direct measure of reproductive fitness. They obtained data on sperm quantity, motility, and density from a health study of 425 Vietnam war veterans aged 31 to 44 and compared them with results of the vets' intelligence tests. After correcting for factors such as drug use, they found a low but still significant (.13) correlation, roughly the same as that for height and IQ (.15).

Psychologist Todd Shackelford of Florida Atlantic University, Davie, is skeptical about a general fitness factor. He says there is no evidence for a correlation between sperm quality and left-right symmetry, a measure of health and physical quality, and only small relationships have been found between such measures and intelligence. Nonetheless, he says, it's "certainly an interesting area of work worth pursuing." **–C.H.**



LETTERS

edited by Jennifer Sills

Retraction

EXTENSIVE EFFORTS WITH REVAMPED APPARATUS TO REPRODUCE THE RESULTS PUBLISHED IN OUR 2004 *Science* Report, "Real-time quantum feedback control of atomic spin-squeezing" (*1*), have failed, as have attempts to develop a quantitative understanding of how those results could have arisen spuriously. We must therefore retract the Report.

J. M. Geremia accepts primary responsibility for his large role in acquiring and analyzing the data upon which this paper was based. J. K. Stockton and H. Mabuchi also accept responsibility for failing initially to probe these results with sufficient skepticism. In the course of our efforts to understand the results subsequent to their publication, we have come to appreciate that analyzing Faraday spectroscopy of alkali clouds at high optical depth in precise quantitative detail is surprisingly challenging. Our understanding of this system as of 2006 is described in J. K. Stockton's thesis (*2*), together with a preliminary reassessment of published results; continuing research in the group of H. Mabuchi has improved upon the thesis results. We now have a technical understanding sufficient to rule out any possibility of spin-squeezing under the conditions of our 2004 experiment.

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2. J. K. Stockton, thesis, California Institute of Technology, Pasadena, CA (2006); http://resolver.caltech.edu/CaltechETD:etd-02172007-172548.

Doubts About GM Crops

N. FEDOROFF'S EDITORIAL ("SEEDS OF A PERfect storm," 25 April, p. 425) calls on the scientific community to rally around genetically modified (GM) crops as the basis for "a second Green Revolution." She asks whether we have "the will and the wisdom" to lower regulatory barriers and integrate these crops fully into world agriculture. However, her framing of the issue is dangerously narrow.

First, the decision about whether and how to deploy GM crops is not just about safety. There are two imperatives for 21st-century agriculture: to increase yields and to fully integrate agriculture with biodiversity conservation and the provisioning of ecosystem services. Fortunately, it appears possible to do both (*1*), but it is not clear what role GM crops can or should play in an agriculture designed for systemic benefits and not just for maximizing the yield of monocrops.

GM crops

CREDIT: ORJAN F. ELLINGVAG/DAGENS NARINGSLIV/CORBIS

Second, consumers are correct to be skeptical about a regulatory process in which the corporations being regulated have extraordinary influence. In this context, to simply appeal to the fait accompli of a quarter-century's experience and a billion acres on the ground is naïve. As Marvier *et al*. point out in the same issue (*2*), despite more than a billion acres, we still don't have a full accounting of the ecological costs (or benefits) of these crops.

Finally, Fedoroff presents no evidence that GM crops are the key to solving our looming global food supply issues. A recent international assessment (IAASTD) concluded that they are "appropriate in some contexts, unpromising in others, and unproven in many more" (*3*). Although the IAASTD has its critics, it appears to be the best starting point we have for getting past the narrow self-interest of agribusiness corporations. Molecular biologists who sincerely wish to see their efforts serve humanity would be wise to treat the public as a full and respected partner in this debate.

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2. M. Marvier *et al*., *Science* **320**, 452 (2008).
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Italy Not Alone in Science System Woes

IN THE LETTER "OPEN LETTER TO SENATOR Rita Levi-Montalcini" (21 March, p. 1615), R. Clementi *et al*. raise concerns regarding the paucity of long-term contracts among medical scientists in Italy.

It is true that the nonmeritocratic system causes many young researchers to fail. The system is further jeopardized by the fact that those who do achieve a "professor" position can hold the position (with its financial guarantee) for years regardless of productivity. Professors can become "empty names"—they are hardly reachable and sometimes they are not even there. Even worse, there are no formal procedures to handle misconduct allegations in Italian universities.

However, these challenges are not unique to the Italian research community. In the United States, graduates also hold temporary

positions for many years before the possibility of attaining even the nontenured rank of assistant professorship. At this phase, renewal of contract depends mostly on their productivity (i.e., the number of publications). In most institutions, investigators are required to obtain funding to support their salaries, and this has become more challenging, given the recent drop in success rates for NIH-funded grant applications (*1*). The candidate is eligible for tenure only after a long probationary period of at least 7 years and, according to one study (*2*), only 50% of the candidates will obtain tenure, at which point their average age is 43 to 44 (*3*).

Although presenting some disturbing characteristics, in comparison to the United States, Italy does not seem to represent such a dramatic case.

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1. Office of Extramural Research (http://grants.nih.gov/grants/award/success.htm).
2. M. J. Dooris, M. Guidos, paper presented at the *Annual Forum of the Association for Institutional Research*, Chicago, IL, 14 to 18 May 2006 (www.psu.edu/president/pia/planning_research/reports/AIR_Tenure_Flow_Paper_06.pdf).
3. National Study of Postsecondary Faculty (http://nces.ed.gov/dasolv2/tables).

Leave Regulation to the FDA

FORMER FDA COMMISSIONER DONALD KENNEDY recently opined ("Misbegotten preemptions," Editorial, 2 May, p. 585) that makers of FDA-regulated products should be subject to lawsuits in state courts when things go wrong, even if they have followed FDA's rules to the letter. I disagree. The regulatory process set up by Congress for the testing and approval of these products is, although imperfect, still the gold standard. It is clearly superior to regulation through ad hoc litigation. Lawsuits to penalize companies when an unknown risk results in harm to a patient do so only by undermining FDA's authority to decide which risks are significant and which are not.

What would Kennedy's new regulatory regime look like? We can make an educated guess from the experience that every one of us has faced in applying for a loan. Those pages of disclosure, opaque and endless, are the lenders' rational response to the possibility of litigation.

Kennedy argues that regulation by jury is needed to supplement the FDA's imperfect ability to detect and manage risks in medical products. He seems to ignore the continuing growth of FDA's considerable capabilities. Every drug candidate today has to survive regulatory scrutiny on dimensions of safety that even 10 years ago could not have been identified. We know more about the safety profiles of our medicines than ever before. This is the result of deeper biological understanding, improved methods of gathering and interpreting information about patient experiences (including large observational and epidemiological studies), and strengthened regulatory requirements for monitoring and reporting safety data after a product is approved. Risk-management plans for the entire product life cycle are rapidly becoming the norm.

Few older drugs, even aspirin, would make it through this gauntlet today. Evidence of the benefits of modern safety testing can be found in recent work by Frank Lichtenberg, National Bureau of Economic Research (*1*), which studied over half a million patients and the impact of the vintage (original FDA approval date) of the drugs they took on their 3-year probability of survival. It found that patients on the oldest drugs (pre-1970 approval) did the worst, with 4.4% actual mortality compared to 3.7% actual mortality for patients on drugs with approval after that date. That is a 16% decrease in actual mortality. The trend was sustained by a decade of approval, with the newest vintage drugs (approval after 1990) doing the best. These statistics suggest that newer is better, that pharmaceutical and medical standards are improving, and that the FDA is raising its game.

The FDA's regulatory system will never be perfect, but the greater danger to public health is that we deter innovation by trying to regulate drug development through litigation. If the FDA is to be the authority on what the label says, juries and judges cannot be given license to rewrite the label and thereby regulate the FDA. Any honest trial lawyer will tell you that litigation is fueled by emotion, theatrics, and opportunism. It does not produce predictable or systematic results. It certainly does not produce science, and it should not be allowed to replace scientific judgment.

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Letters to the Editor

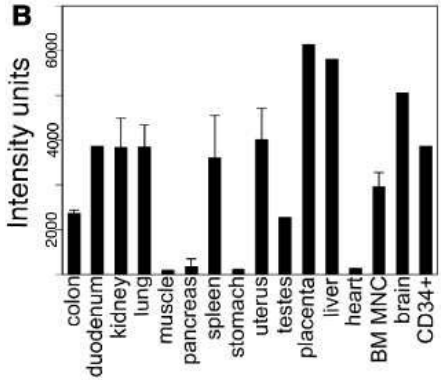
Letters (~300 words) discuss material published in *Science* in the previous 3 months or issues of general interest. They can be submitted through the Web (www.submit2science.org) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.

Reference

1. Frank R. Lichtenberg, "The effect of drug vintage on survival: Micro evidence from Puerto Rico's Medicaid Program" (National Bureau of Economic Research, NBER Working Paper No. 10884, Cambridge, MA, 2004); www.nber.org/papers/w10884.

CORRECTIONS AND CLARIFICATIONS

Reports: "A heme export protein is required for red blood cell differentiation and iron homeostasis" by S. B. Keel *et al.* (8 February, p. 825). Three editorial errors were made. The *y*-axis label for Fig. 2B should read CD71 (not CD17). The corrected Fig. 3B is shown here; stomach, the label for the eighth bar, has been restored. In the legend to Fig. 4, the concentration of hepcidin should read 1 μg/ml.



TECHNICAL COMMENT ABSTRACTS

Comment on "Major Australian-Antarctic Plate Reorganization at Hawaiian-Emperor Bend Time"

Anahita A. Tikku and Nicholas G. Direen

Whittaker *et al.* (Reports, 5 October 2007, p. 83) presented reconstructions for Australia and Antarctica showing a change in relative plate motion ~53 million years ago, coincident with an inferred major global plate reorganization. This comment addresses problematic areas in their assumptions and the geological consequences of their reconstructions.

Full text at www.sciencemag.org/cgi/content/full/321/5888/490c

Response to Comment on "Major Australian-Antarctic Plate Reorganization at Hawaiian-Emperor Bend Time"

J. M. Whittaker, R. D. Müller, G. Leitchenkov, H. Stagg, M. Sdrolias, C. Gaina, A. Goncharov

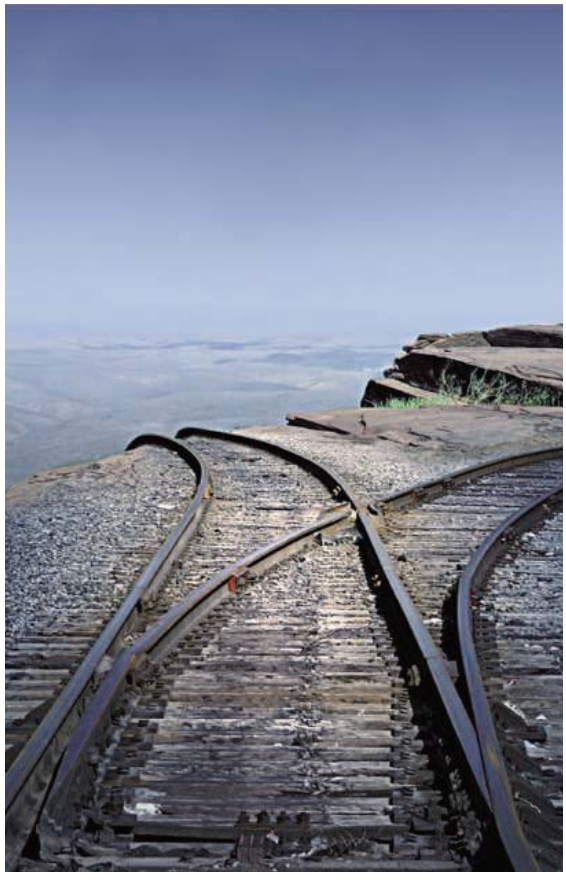
Accurately locating boundaries between continental and oceanic crust is topical in view of locating offshore boundaries relevant to margin formation models, plate kinematics, and frontier resource exploration. Although we disagree with Tikku and Direen's interpretations, the associated controversies reflect an absence of agreed-upon geophysical criteria for distinguishing stretched continental from oceanic crust, and lack of samples from nonvolcanic margins.

Full text at www.sciencemag.org/cgi/content/full/321/5888/490d

TECHNOLOGY AND SOCIETY

Avoiding a Cliff Dive

Brett M. Frischmann



CREDIT: IVO VAN DER ENT/COURTESY JONATHAN ZITTRAIN

The image on the cover of Jonathan Zittrain's *The Future of the Internet: And How to Stop It* depicts train tracks that fork at the edge of a cliff. One branch falls off a cliff to the left; the other heads off safely into the distance. Like the artwork, this compelling book starkly presents an impending decision: essentially the choice (of grand social consequence) between two futures for the Internet and the information, computing, and communications ecosystem it has generated. Zittrain (a professor at Harvard Law School) contends that we are approaching rapidly the junction where a choice—really, a series of choices—must be made. And there are many obstacles to choosing the path to salvation. We need to recognize that we are on the train and see where it is heading. We next need to identify the correct path, and then we must make the appropriate decisions that will allow us to follow it. Failure to do so means heading off the side of a cliff to our doom. Though perhaps a bit exaggerated, Zittrain's framing is powerful.

The Future of the Internet
And How to Stop It
by Jonathan L. Zittrain

Yale University Press, New Haven, CT, 2008. 350 pp. $30. ISBN 9780300124873. (*1*)

A prominent feature of his framing, repeated throughout the book, is the dichotomy between generative and sterile technologies. At the outset, Zittrain uses two technologies developed by Apple to illustrate this idea. First, he describes the Apple II personal computer as a "quintessentially *generative* technology" because it was a "platform," it "invited people to tinker with it," "Jobs (and Apple) had no clue how the machine would be used," it was "designed for surprises," and fortunately, nothing constrained the personal computer to the "hunches of the founders." At the opposite extreme, he tells us, is the Apple iPhone, which is "sterile" because it "comes preprogrammed"; is not a platform for user innovation; its "functionality is locked in"; and only Apple-authorized innovation is permitted.

Zittrain defines generativity as "a system's capacity to produce unanticipated change through unfiltered contributions from broad and varied audiences." He elaborates on the concept and outlines five factors (leverage, adaptability, ease of mastery, accessibility, and transferability) that indicate whether a technology or system is more or less generative. Over the course of the book, he uses the term as an adjective for technologies, systems, human behaviors, tools, and much more. It seems to encompass (and to some degree, conflate) a number of characteristics studied by scholars of technological innovation, including the degree to which something is open or closed (in terms of access and conditions on use), the degree to which something is general or special purpose (in terms of functionality or utility), whether innovation is centralized or decentralized, ease of use, and design complexity. Nonetheless, the generative-sterile dichotomy works well; it focuses the reader's attention acutely on a key functional attribute: openness to innovation by users (*2*).

The author uses his description of the differences between the Apple II and the iPhone to set up his predictions about the future of the Internet:

> In the arc from the Apple II to the iPhone, we learn something important about where the Internet has been, and something more important about where it is going. The PC revolution was launched with PCs that invited innovation by others. So too with the Internet. Both were generative: they were designed to accept any contribution that followed a basic set of rules (either coded for a particular operating system, or respecting the protocols of the Internet). Both overwhelmed their respective proprietary, nongenerative competitors, such as the makers of stand-alone word processors and proprietary online services like CompuServe and AOL. But the future unfolding right now is very different from this past. The future is not one of generative PCs attached to a generative network. It is instead one of sterile *appliances* tethered to a network of control.

Critically, Zittrain puts the personal computer front and center. This is a substantial contribution to the debate about the future of the Internet. Oddly, scholars and policy-makers have all but ignored the importance of the personal computer. "Too often, a discussion of the Internet and its future stops just short of its endpoints, focusing only on the literal network itself: how many people are connected, whether and how it is filtered, and how fast it carries data. These are important questions, but they risk obscuring the reality that people's experiences with the Internet are shaped at least as much by the devices they use to access it." [On filtering, another important issue of network access and quality, see (*3*).] The personal computer is a general-purpose technology that has served us incredibly well as the key platform for software utilization and innovation and as the primary device people use to communicate on the Internet. Economists have recognized the importance of general-purpose technologies (such as the personal computer) and infrastructure (such as the Internet) for economic development and growth. [The exact relationships between general-purpose technologies, infrastructure, and economic growth are subjects of intense study within economics; see, for example, (*4*, *5*).] To fully grapple with many difficult issues and to

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better account for the potential long-term consequences, we need to consider more carefully what the future may hold for the personal computer and the Internet in tandem.

Simply put, Zittrain's thesis is that although the Internet and personal computer are generative, both are at risk of becoming sterile. Paradoxically, the reason why both are at risk is their generativity. The very openness to unanticipated, unfiltered changes and innovation gives rise to the pressures for more controlled environments. A generative system does not mean that all changes or innovations will be good. Quite to the contrary, malicious spyware and computer viruses ("bad code"), among many other nasty things, emerge and proliferate alongside the beneficial innovations fostered in the uncontrolled and chaotic environment of generative personal computers and a generative Internet. Furthermore, the more visible, salient, and disruptive the bad code is, the less consumers and businesses appreciate the good and tolerate the chaos from which it came.

As such, for Zittrain the evolution of the Internet to a "network of control" and of personal computers to sterile appliances is underway. The former is seen in the current battle over network neutrality; the latter, in the proliferation of various "locked-down" end-user devices, which he calls "information appliances" (such as "mobile phones, video game consoles, TiVos, iPods, and BlackBerries"). Because consumers often are ill-equipped to deal with most of the harmful stuff—and, in fact, are often to blame because of "their own surfing or program installation choices"—they will increasingly look for gatekeeping, security, and regulation in the devices, technologies, and services they purchase. To better satisfy this demand and also enable devices to "call home" for updates, the appliances may be "tethered" to their suppliers by an Internet connection. This shift sterilizes the Internet and the personal computer, ostensibly at the consumer's request.

Yet Zittrain contends that even though sterilization might be responsive to consumer demand for a more stable and secure computing environment and prioritized services, it is undesirable. He offers three arguments to support this contention: The loss of generativity would affect innovation. Tethered appliances coupled with a network of control dramatically increase regulation of end-user behavior (by government and companies). And, based on his detailed discussion of Wikipedia, he suggests that generativity supports widespread participation and cooperation in cultural production and governance.

Zittrain's arguments that we ought to preserve generative personal computers and the generative Internet are provocative but seem incomplete. He recognizes that there would be opportunity costs but does not fully explain why generativity is worth preserving despite these costs. He does not engage in a structured analysis of the tradeoffs. His appeal to innovation, for example, seems insufficient because he makes no specific theoretic or empirical claims about the quality or quantity of innovation under different degrees of generativity. Such claims could and should be developed but, in fairness, require considerably more work (*6*). Zittrain's argument about increased regulation of user behavior is powerful, but it depends on his prediction of a complete shift to tethered appliances and controlled networks. To the extent that alternatives persist or hybrid scenarios emerge, it is not clear that his worst fears would materialize. (Of course, this means that sustaining alternatives is important.) Finally, with respect to his third argument, the relationship between generativity and participation and cooperation seems somewhat circular or, at least, underspecified (*7*). Though incomplete, these arguments merit serious attention and further development.

Zittrain make a number of recommendations about how to avoid heading off the cliff. One interesting suggestion involves porting and reconfiguring tools used at the content layer to encourage collective action—of the sort employed by Wikipedia and eBay, for example—to solve "bad code" problems and bring some stability to the Internet. That is, empower users to become part of the solution. Zittrain discusses a project he is involved with called StopBadware that is "designed to assist rank-and-file Internet users in identifying and avoiding bad code." The project allows users to contribute data about code running on their personal computers and potentially identify and mitigate security threats collectively. He also discusses a partnership between Google and StopBadware that identifies Web sites that have malicious code hidden in them and provides a warning to users in their search results. These are promising and innovative steps.

Whether these and the other prescriptions Zittrain discusses are necessary (or sufficient) to avoid a cliff dive is probably beside the point. The future of the Internet cannot be stopped, and it is unlikely to be either of the two extremes the author describes. He acknowledges this and begins to consider emergent hybrids that may involve the best or worst of the extremes. Most important, *The Future of the Internet* identifies and analyzes many of the key issues, obstacles, and tradeoffs that will define our future.

References and Notes

1. The book can be read and commented on at http://yupnet.org/zittrain/.
2. There is a rich literature on user innovation, e.g., E. von Hippel, *Democratizing Innovation* (MIT Press, Cambridge, MA, 2005).
3. R. Deibert, J. Palfrey, R. Rohozinski, J. Zittrain, Eds., *Access Denied: The Practice and Policy of Global Internet Filtering* (MIT Press, Cambridge, MA, 2008).
4. T. Bresnahan, M. Trajtenberg, *J. Economet.* **65**, 83 (1995).
5. P. Aghion, P. Howitt, *Endogenous Growth Theory* (MIT Press, Cambridge, MA, 1998).
6. This is a complex task. At either extreme, or anywhere in between, on the generativity scale (assuming such a scale could be constructed), innovation will occur; we should expect different profiles of investments, participants, objectives and motivations, innovations, and so on. Society might value the type of innovation that results from generative systems, but to what degree? At what cost? Compared to what? Etc. Zittrain does a good job of setting the stage for these questions.
7. For a recent examination of how the Internet enables user participation and cooperation in a variety of contexts and why encouraging such activities is socially valuable, see Y. Benkler, *The Wealth of Networks: How Social Production Transforms Markets and Freedom* (Yale Univ. Press, New Haven, CT, 2006); reviewed by B. Frischmann, *Univ. Chicago Law Rev.* **74**, 1083 (2007).

10.1126/science.1160394

GENETICS

Thoughts on Humane Genetics

Robert Pollack

Archibald Garrod, M.D., opened the field of medical genetics with his 1902 *Lancet* article, "The incidence of Alkaptonuria: A study in chemical individuality" (*1*). He first reported that the offspring of cousins expressed the phenotype of this genetic variant at a high frequency. From there, he leaped to the testable predictions that humans, like Mendel's peas, inherited this chemical difference as a pair of discrete "Mendelian characters" and that both parents had to contribute the variant "character" for the offspring to show the difference. Garrod saw even further than what we would call a recessive phenotype: he closed his paper with the insight that genetic variation of chemical structures from one person to another might explain the differences among us in many—perhaps all—other aspects of our appearance and behavior. His

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prescience was, however, not entirely modern. He had no explanation for the initial presence of any chemical genetic variation, and Darwinian natural selection was not in his toolkit of ideas.

Within a decade of Garrod's paper, the notion of scientific and medical intervention for the sake of improvement of the species or—as it was then called with portentous ambivalence—the "race," emerged fully formed under the flag of Francis Galton's neologism "eugenics." Could it not be that with our intellect, we might take charge of our species' future, weeding out some hereditable variants and seeding others so that humans as a whole might be quickly improved or even perfected? For seeding, or "positive eugenics," Mendelian genetics was seen by the eugenics movement as the source of advice for the guidance of young people about to marry and have babies. For weeding, or "negative eugenics," the genetic interpretation of family trees was to be the scientific rationale in the United States for governments, in service to the race, to examine, catalog, sterilize, incarcerate, and institutionalize large numbers of men, women, and children.

Charles Davenport was the guiding light of American eugenics, both positive and negative. In 1910, with funds from the widow of railway magnate E. H. Harriman, he established the Eugenics Record Office at Cold Spring Harbor, New York. There he and his superintendent Harry H. Laughlin kept files on hundreds of thousands of family trees, counseled the wealthy on how to protect their fortunes from bad germ plasm, wrote model eugenics laws at the request of state and federal authorities, and maintained warm collegial relations with the burgeoning eugenics movement in interwar Germany.

In 1911, Davenport laid out the agenda for American eugenics in *Heredity in Relation to Eugenics* (*2*), which became the standard textbook in U.S. universities. *Davenport's Dream* provides a facsimile of that edition, buttressed by short commentaries on topics that interested Davenport (such as the meaning of genetic variation, mental illness, nature versus nurture, and human evolution) from 12 leading genetics researchers. The volume, edited by two current members of Cold Spring Harbor Laboratory, Jan Witkowski and John Inglis, is important: well worth reading and well worth having for classroom instruction at any level from high school through graduate school, divinity school, medical school, or law school.

Davenport's Dream
21st Century Reflections on Heredity and Eugenics

by Jan A. Witkowski and John R. Inglis, Eds.

Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 2008. 520 pp. $55. ISBN 9780879697563.

CREDIT: AMY POLLACK

I was somewhat surprised by the publication of this volume. When I was a senior scientist at the Cold Spring Harbor Laboratory in the early 1970s, Davenport and eugenics were not topics people there were interested in discussing—for good reason. Davenport's Eugenics Record Office contributed the scientific rationale for the restrictive U.S. immigration laws of 1924, which made it very difficult for those members of what Davenport called "the Hebrew Race" to escape to America from Nazi Germany a decade later. Approaching the book, I could not forget this nor the fact that my and my wife's ancestral families were for the most part killed in the Holocaust.

Yet because of the essays, I am pleased to recommend the volume to the broadest possible audience. Davenport's reprinted text may be thought of as an example of one of Stephen Gould's evolutionary "spandrels," that is, a scaffolding of now-useless information on which is built a novel and viable structure. In this case, the current viable and valuable structure is the set of commentaries. These elegantly summarize the state of medical genetics today, touching on aspects such as the Human Genome Project, ex vivo technologies of genetic selection, intentional variation and quick detection by reverse genetics, and the emerging understanding of the vast complexity of RNA-driven gene regulation by noncoding regions that rarely expresses itself as a "single gene" phenotype. The crucial question remains: in light of the disasters of eugenics, what is the proper use of what we know about human DNA?

The essays (especially those by Maynard Olson and Douglas Wallace, the editors' introduction, and James Watson's personal reflections) provide a firm foundation for answering that question: We are the products of natural selection working on inevitable, unavoidable genetic variation. That variation will never cease. The broadest possible definition of "normal" is the one closest linked to the realities of natural selection. No one can say which (if any) human genetic variants will survive the anthropocene epoch (*3*, *4*) we have just entered. Therefore eugenics was and remains a dead end, and it cannot be the answer.

Furthermore, whereas genetic science must not be used as an excuse to torture, punish, or in any way hurt a person, the findings from human genetics should be made available to alleviate individual suffering. Although eugenics proved a disaster, medical genetics has from its beginnings been a gift of the intellect from which every human might benefit—but the potential gains must be considered only one person at a time and, each time, only for that individual's benefit. Such a use of genetics does not attempt to imitate (and sharpen) Darwinian natural selection as eugenicists did. Instead, it intends to ameliorate natural selection's harshest decrees on members of our species. In laying out that difference with clarity and rigor, *Davenport's Dream* is a gift as well.

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2. C. B. Davenport, *Heredity in Relation to Eugenics* (Holt, New York, 1911).
3. P. J. Crutzen, E. F. Stoemer, *IGBP Newsletter* no. 41, 17 (2000).
4. J. Zalasiewicz *et al.*, *GSA Today* **18**, 4 (February 2008).

10.1126/science.1160234

DIVERSITY

Gender Similarities Characterize Math Performance

Standardized tests in the U.S. indicate that girls now score just as well as boys in math.

Janet S. Hyde,[1]* Sara M. Lindberg,[1] Marcia C. Linn,[2] Amy B. Ellis,[3] Caroline C. Williams[3]

Gender differences in mathematics performance and ability remain a concern as scientists seek to address the underrepresentation of women at the highest levels of mathematics, the physical sciences, and engineering (*1*, *2*). Stereotypes that girls and women lack mathematical ability persist and are widely held by parents and teachers (*3*–*5*).

Meta-analytic findings from 1990 (*6*, *7*) indicated that gender differences in math performance in the general population were trivial, $d = -0.05$, where the effect size, d, is the mean for males minus the mean for females, divided by the pooled within-gender standard deviation. However, measurable differences existed for complex problem-solving beginning in the high school years ($d = +0.29$ favoring males), which might forecast the underrepresentation of women in science, technology, engineering, and mathematics (STEM) careers.

Grade	$d \pm$ SE	Variance ratio	*N*
Grade 2	0.06 ± 0.003	1.11	460,980
Grade 3	0.04 ± 0.002	1.11	754,894
Grade 4	−0.01 ± 0.002	1.11	763,155
Grade 5	−0.01 ± 0.002	1.14	929,155
Grade 6	−0.01 ± 0.002	1.14	886,354
Grade 7	−0.02 ± 0.002	1.16	898,125
Grade 8	−0.02 ± 0.002	1.21	837,979
Grade 9	−0.01 ± 0.003	1.14	608,229
Grade 10	0.04 ± 0.003	1.18	619,591
Grade 11	0.06 ± 0.003	1.17	446,381

Effect sizes across grades for U.S. mathematics tests; results are similar across grades 2 through 11.

Percentage of children scoring above indicated percentile and ratios						
Ethnic group	Above 95th percentile			Above 99th percentile		
	F	M	M/F	F	M	M/F
Asian/Pacific Islander (*n* =219)	5.71	6.27	1.09	1.37	1.25	0.91
White (*n* = 3473)	5.38	7.80	1.45	0.90	1.85	2.06

The upper tail. Percentage of Minnesota children scoring above the 95th and 99th percentiles in 11th grade mathematics testing, by gender and ethnicity. Too few students scored above the 95th percentile to compute reliable statistics for these groups: American Indians, Hispanics, and Black not Hispanic.

Since this study of data from the 1970s and 1980s, several crucial cultural shifts have occurred that merit a new analysis of gender and math performance. In previous decades, girls took fewer advanced math and science courses in high school than boys did, and girls' deficit in course taking was one of the major explanations for superior male performance on standardized tests in high school (*8*). By 2000, high school girls were taking calculus at the same rate as boys, although they still lagged behind boys in the number of them taking physics (*9*). Today, women earn 48% of the undergraduate degrees in mathematics, although gender gaps in physics and engineering remain large (*10*).

Contemporary state assessments. State assessments of cognitive performance provide a contemporary source of data on these questions. Many states have conducted assessments for years, but with the advent of No Child Left Behind (NCLB) legislation, all states are mandated to conduct such assessments annually. This testing provides an exceptional opportunity to analyze current gender differences in math performance, particularly because of the extraordinary number of test takers.

Although NCLB requires states to post test results publicly, few states report data by gender and, of those that do, fewer report the necessary statistical information to compute effect sizes. Therefore, we contacted the state departments of education of all 50 states, requesting detailed statistical information on gender differences, by grade level and by ethnicity. Responses with adequate statistical information were received from 10 states: California, Connecticut, Indiana, Kentucky, Minnesota, Missouri, New Jersey, New Mexico, West Virginia, and Wyoming. In all cases, the data represent the testing of all students attending school in that grade. These states are geographically diverse and appear to be representative of all 50 states insofar as their average scores on the National Assessment of Educational Progress (NAEP, a federal assessment that carefully samples students nationwide) match the average for all 50 states quite closely. For 8th-graders, the average NAEP mathematics score was 280.22 for our 10 states and 280.17 for all 50 states (*11*).

Gender and average performance. Effect sizes for gender differences, representing the testing of over 7 million students in state assessments, are uniformly <0.10, representing trivial differences (see table, top left, and table S1). Of these effect sizes, 21 were positive, indicating better performance by males; 36 were negative, indicating better performance by females; and 9 were exactly 0. From this distribution of effect sizes, we calculate that the weighted mean is 0.0065, consistent with no gender difference (see chart on p. 495 and fig. S1). In contrast to earlier findings, these very current data provide no evidence of a gender difference favoring males emerging in the high school years; effect sizes for gender differences are uniformly <0.10 for grades 10 and 11 (see table, top left, and table S1). Effect sizes for the magnitude of gender differences are similarly small across all ethnic groups (table S2). The magnitude of the gender difference does not exceed $d = 0.04$ for any ethnic group in any state.

Gender and variance. Another explanation for the underrepresentation of women at the highest levels in STEM careers has focused not on averages, but on variance, the extent to which scores of one gender or the other vary from the mean score. The hypothesis that the variability of intellectual abilities is greater among males than among females and produces a preponderance of males at the highest levels of performance was originally proposed over 100 years ago (*12*).

The variance ratio (VR), the ratio of the male variance to the female variance, assesses

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these differences. Greater male variance is indicated by VR > 1.0. All VRs, by state and grade, are >1.0 [range 1.11 to 1.21 (see top table on p. 494)]. Thus, our analyses show greater male variability, although the discrepancy in variances is not large. Analyses by ethnicity show a similar pattern (table S2).

Does this greater variability translate into gender differences at the upper tail of the distribution (*13*)? Data from the state assessments provide information on the percentage of boys and girls scoring above a selective cut point. Results vary by ethnic group. The bottom table on p. 494 shows data for grade 11 for the state of Minnesota. For whites, the ratios of boys:girls scoring above the 95th percentile and 99th percentile are 1.45 and 2.06, respectively, and are similar to predictions from theoretical models. For Asian Americans, ratios are 1.09 and 0.91, respectively. Even at the 99th percentile, the gender ratio favoring males is small for whites and is reversed for Asian Americans. If a particular specialty required mathematical skills at the 99th percentile, and the gender ratio is 2.0, we would expect 67% men in the occupation and 33% women. Yet today, for example, Ph.D. programs in engineering average only about 15% women (*14*).

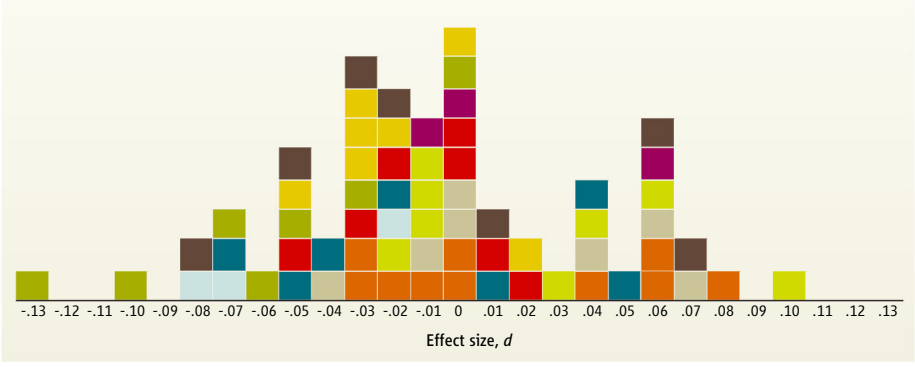
Gender and item complexity. An additional issue in assessing gender differences in math performance and the underrepresentation of women in STEM careers is the question of the cognitive complexity or depth of knowledge being tested. Earlier studies (*6*) indicated that, although girls equaled or surpassed boys in basic computation and understanding of mathematical concepts, boys exceeded girls in complex problem-solving beginning in the high school years, $d = +0.29$. Complex problem-solving is crucial for advanced work in STEM careers. At the time of the 1990 meta-analysis, girls were less likely to take advanced math and science courses, and this gender difference in course choice was a likely explanation for the gender gap in complex problem-solving (*8*).

Today, with the gender gap erased in taking advanced math courses, does the gender gap remain in complex problem-solving? To answer this question, we coded test items from all states where tests were available, using a four-level depth of knowledge framework (*15*). Level 1 (recall) includes recall of facts and performing simple algorithms. Level 2 (skill/concept) items require students to make decisions about how to approach a problem and typically ask students to estimate or compare information. Level 3 (strategic thinking) includes complex cognitive demands that require students to reason, plan, and use evidence. Level 4 (extended thinking) items require complex reasoning over an extended period of time and require students to connect ideas within or across content areas as they develop one among alternate approaches. We computed the percentage of items at levels 3 or 4 for each state for each grade, as an index of the extent to which the test tapped complex problem-solving. The results were disappointing. For most states and most grade levels, none of the items were at levels 3 or 4. Therefore, it was impossible to determine whether there was a gender difference in performance at levels 3 and 4.

The dearth of level-3 or level-4 items in state assessments has an additional serious consequence. With the increased emphasis on testing associated with NCLB, more teachers are gearing their instruction to the test (*16*). If the tests do not assess the sorts of reasoning that are crucial to careers in STEM disciplines, then these skills may be neglected in instruction, putting American students at a disadvantage relative to those in other countries where tests and curricula emphasize more challenging content (*17*).

To address this limitation in the state assessments, we returned to the NAEP data (*18*). NAEP categorizes items as easy, medium, or hard. We coded hard sample items for depth of knowledge. No items were at level 4 but many were at level 3. We computed the magnitude of gender differences on the hard items that were at level 3 depth of knowledge. At grade 12, effect sizes for these items ranged between 0 and 0.15 (average $d = 0.07$). At grade 8, effect sizes for these items ranged between 0 and 0.08 (average $d = 0.05$). Thus, even for difficult items requiring substantial depth of knowledge, gender differences were still quite small.

Conclusion. Our analysis shows that, for grades 2 to 11, the general population no longer shows a gender difference in math skills, consistent with the gender similarities hypothesis (*19*). There is evidence of slightly greater male variability in scores, although the causes remain unexplained. Gender differences in math performance, even among high scorers, are insufficient to explain lopsided gender patterns in participation in some STEM fields. An unexpected finding was that state assessments designed to meet NCLB requirements fail to test complex problem-solving of the kind needed for success in STEM careers, a lacuna that should be fixed.



Effect sizes across grades and U.S. states. The weighted mean is 0.0065, consistent with no gender difference. Each square represents the effect size for one grade within one state. New Mexico (pea green), Kentucky (pink), Wyoming (dark brown), Minnesota (teal), Missouri (red), West Virginia (gold), Connecticut (tan), California (orange), Indiana (yellow), New Jersey (purple).

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21. We thank personnel in each of the 10 responding states for providing data. Special thanks to Margaret Biggerstaff of the Minnesota Department of Education for additional analyses. This research was funded through grant REC 0635444 from NSF. Any findings, conclusions, or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation.

10.1126/science.1160364

Supporting Online Material
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MOLECULAR BIOLOGY

RNA Interference in the Nucleus

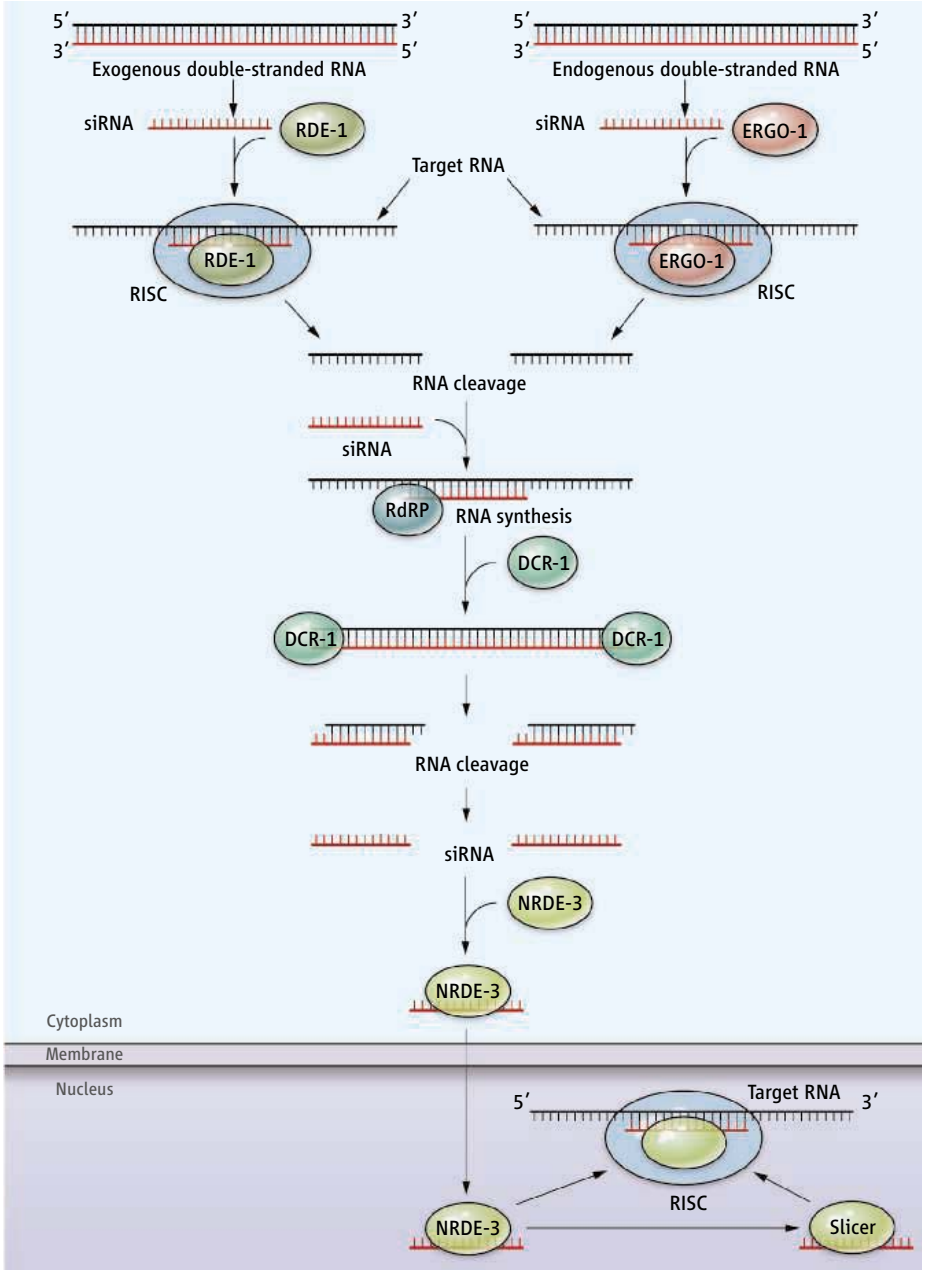
Studies of a protein that shuttles small regulatory RNA molecules into the nucleus suggest that additional factors may be required for nuclear import.

Gunter Meister

RNA-guided gene silencing pathways use double-stranded RNA (dsRNA) to inhibit gene expression in a sequence-specific manner. In RNA interference (RNAi), dsRNA is processed to small interfering RNAs (siRNAs), which guide the cleavage of complementary RNA molecules by the RNA-induced silencing complex (RISC) (*1*). Members of the Argonaute protein family are the direct binding partners of siRNAs and form the core of RISC (*2*, *3*). Although RNAi predominantly occurs in the cell's cytoplasm, nuclear RNAs can be targeted by siRNAs as well. A study by Guang *et al*. on page 537 of this issue now reports the identification of an Argonaute protein in the worm *Caenorhabditis elegans* that is required for nuclear siRNA import and is therefore essential for nuclear RNAi (*4*).

Through a genetic screen, Guang *et al*. determined that mutations in the gene *nrde-3* (*nuclear RNAi defective-3*) resulted in animals that no longer perform nuclear RNAi (cytoplasmic RNAi was unaffected). NRDE-3 is one of the 27 Argonaute proteins in *C. elegans*, but its function had not been characterized. The authors found that NRDE-3 exclusively binds to endogenous siRNAs, a class of small RNAs that regulates gene expression and requires the function of the exonuclease ERI-1, and RRF-3, an RNA-dependent RNA polymerase (*5*, *6*). A considerable portion of the identified siRNAs originates from the open reading frame E01G4.5. Indeed, the amount of precursor messenger RNA corresponding to E01G4.5 was elevated in *nrde-3* mutant animals, indicating that NRDE-3 is required for nuclear silencing of the endogenous siRNA target E01G4.5. However, endogenous siRNAs are also present in *nrde-3* mutants. Thus, this class of small RNAs may associate with other Argonaute proteins as well, because mature small RNAs most likely require Argonaute association for stability. In mutant animals that are defective in endogenous siRNA production, NRDE-3 could associate with exogenous siRNAs, indicating that NRDE-3 interacts with siRNAs independently of their origin.

NRDE-3 contains an amino acid sequence that encodes a nuclear localization signal, and a fusion protein of NRDE-3 and green fluorescent protein predominantly localized to the nucleus of *C. elegans* cells. Moreover, nuclear localization of NRDE-3 requires siRNA binding, because in a *nrde-3* mutant animal defective in siRNA binding, NRDE-3 did not enter the nucleus. This indicates that the nuclear localization signal alone is not sufficient for



Nuclear interference. (Right) Endogenous siRNAs originating from distinct genomic loci in *C. elegans* guide cleavage of target RNAs by the Argonaute protein ERGO-1. Cleavage products serve as templates for RNA-dependent RNA polymerases (RdRPs) that generate dsRNAs, which are processed by the enzyme DCR-1. **(Left)** Exogenous dsRNA is processed by DCR-1 as well, but RDE-1 serves as the cleavage-competent Argonaute protein. NRDE-3 binds to siRNA products from both pathways and transports them into the nucleus, where an Argonaute protein with Slicer activity facilitates nuclear RNAi. RISC, RNA-induced silencing complex.

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importing NRDE-3 into the nucleus. It is therefore reasonable that siRNA binding might induce conformational changes in the NRDE-3 structure that allow efficient binding of an unknown nuclear import receptor. Vice versa, mutations in the nuclear localization signal of NRDE-3 led to a loss of nuclear RNAi, indicating that NRDE-3 contains a functional nuclear localization signal and that nuclear localization of NRDE-3 is essential for silencing nuclear RNAs.

A large genetic screen in *C. elegans* has previously shown that the Argonaute protein RDE-3 associates with primary siRNAs that directly derive from dsRNA (*7*). Many other Argonaute proteins in *C. elegans* associate with secondary siRNAs that are generated by RNA-dependent RNA polymerases. Consistently, Guang *et al*. found that NRDE-3–bound endogenous siRNAs are most likely produced by RNA-dependent RNA polymerases, which suggests that NRDE-3–mediated nuclear RNAi is a downstream function of primary RNAi events in the cytoplasm (see the figure).

In many organisms, distinct Argonaute proteins are endonucleases that cleave target RNAs with a high degree of complementarity to the bound siRNA (*8*). Structural studies revealed that three catalytic residues within the Piwi domain of Argonaute proteins are important for cleavage activity (*8*). Although NRDE-3 is required for nuclear RNAi, this catalytic triad is not conserved. It may be that NRDE-3 is required only for nuclear import of siRNAs, and that another nuclear Argonaute protein (yet to be identified) might be the mediator of nuclear RNAi. This scenario would involve a transfer of siRNAs from one Argonaute protein to another in the nucleus. Such a transfer process, however, has not yet been observed.

Nuclear functions of siRNAs have been reported in other organisms as well. In humans, siRNAs efficiently silence target RNAs that are exclusively nuclear (*9*). Because the Argonaute protein Ago2 is the only known endonuclease among the human Argonaute proteins, it is very likely that Ago2 mediates nuclear RNAi in human cells. How are human Ago2 and *C. elegans* NRDE-3 imported into the nucleus, and how are these importing pathways regulated? Further studies in this direction will not only lead to a better understanding of the mechanisms underlying nuclear RNAi, but will also show new ways of improving RNAi as a tool for research and therapy.

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10. I thank L. Weinmann and S. Rüdel for critically reading the manuscript. Supported by the Max Planck Society.

10.1126/science.1161854

PHYSICS

Elemental Complexity

Elemental bismuth is generating excitement among condensed matter physicists studying interacting relativistic electrons beyond the quantum limit.

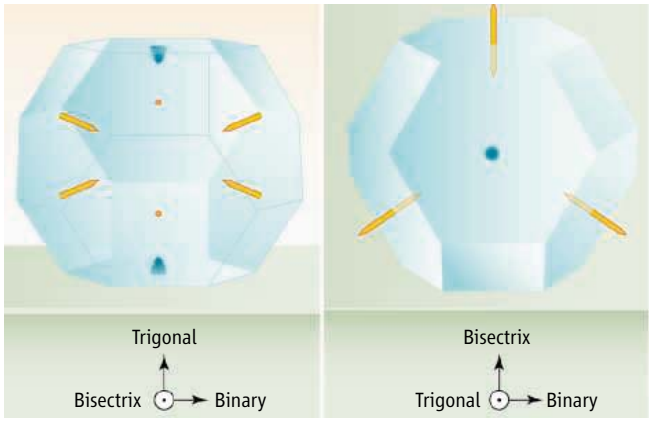
Kamran Behnia

Metals are solids in which conduction electrons remain mobile even at absolute zero temperature. In a semimetal, the concentration of such mobile electrons is extremely low. Whereas in a typical metal, say copper, there is roughly one itinerant electron per atom, in bismuth, the archetypal semimetal, 100,000 atoms share a single mobile electron. On page 547 of this issue, Li *et al.* (*1*) report that in the presence of a strong magnetic field, this dilute electron gas orders in a way never observed in any other material.

The singular role played by bismuth in the history of metal physics owes much to its peculiar Fermi surface (see the figure). Each metal is identified by its Fermi surface set in a Brillouin zone. These objects exist in an imaginary three-dimensional space, called the reciprocal space, where each axis represents the momentum of mobile electrons along each axis of real space. The shape of the Brillouin zone is determined by the crystal structure of the solid in question. The Fermi surface constitutes the borderline between allowed and forbidden electronic states. In many cases, the volume enclosed by the Fermi surface is a "forbidden territory," so that mobile electrons behave as if they were positively charged electrons. Condensed-matter physicists call these electronic quasiparticles "holes."

In the presence of a magnetic field, the movement of electrons is further constrained and confined to quantized orbits known as Landau levels. As the magnetic field increases, these orbits grow in size. As they cross the Fermi surface one after the other, the physical properties of the metal oscillate with magnetic field, a feature dubbed quantum oscillations. The so-called quantum limit is attained when all but the lowest Landau level have passed through the Fermi surface.

In an ordinary metal, the magnetic field required to attain this limit exceeds the fields that humans are currently able to produce by several orders of magnitude. In contrast, in the case of bismuth, when the field is oriented along the high-symmetry crystalline axis known as trigonal, the quantum limit can be attained by a moderate magnetic field currently



Beyond the quantum limit. The tiny Fermi surface of bismuth (dark blue and yellow pockets) occupies a mere 10^{-5} of the Brillouin zone (light blue solid body). The yellow pockets host Dirac fermions. When the field is aligned along the trigonal (**right**), the three yellow ellipsoids are equivalent. Li *et al*. find that when the quantum limit is attained, these Dirac fermions order in a new state.

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available in many laboratories.

It is the tiny size of the Fermi surface in bismuth, itself a direct consequence of the extremely low electron concentration, that makes the quantum limit accessible. The Fermi surface in bismuth consists of a hole ellipsoid with its long axis along the trigonal, and three electron ellipsoids slightly tilted out of the equatorial plane (see the figure). The volume of the hole ellipsoid (which equals the total volume of the three electron ellipsoids) is only 10^{-5} of the Brillouin zone. For a field oriented along the trigonal axis, the cross section of each of these ellipsoids is such that in the presence of a field exceeding 9 T, both electrons and holes are confined to the lowest Landau level.

Thus, bismuth provides a rare opportunity to explore the fate of a three-dimensional electron gas pushed beyond the quantum limit, a subject previously addressed by theorists (*2*) but barely touched by experimentalists. A study on bulk bismuth last year detected unexpected anomalies in transport properties beyond the quantum limit, pointing to unknown many-body effects (*3*). In a two-dimensional electron gas, such effects are known to lead to electron fractionalization (*4*), but little is known about the three-dimensional case. Li *et al.* now present an unprecedented exploration of this limit with a thermodynamic probe and conclude that a phase transition involving electron pockets occurs at the quantum limit.

The electron-like and hole-like carriers in bismuth differ in one important respect. Besides its tiny size, nothing is particularly remarkable about the hole pocket. The other pockets, on the other hand, host Dirac electrons. Such electrons have attracted much attention in the context of research on graphene, a single sheet of graphite (*5*). Dirac electrons do not behave like ordinary mobile electrons confined to a periodic lattice; instead, they share common features with fermions traveling with relativistic velocity in a vacuum.

Li *et al.* performed high-resolution torque magnetometry to probe the electron pockets in bismuth. Their data show anomalies that mark the passage of successive Landau levels through the Fermi level. Just above the quantum limit, the authors detect a set of anomalies, which appear to be the signatures of phase transitions to an unknown new electronic state. Intriguingly, the boundaries of this state tightly encircle the trigonal axis. When the magnetic field is perfectly aligned along the trigonal, the three electron ellipsoids are indistinguishable. In the language of quantum mechanics, there is a threefold degeneracy, which vanishes only when the field is tilted off the trigonal. Li *et al.* boldly suggest that their findings unveil a new collective state of Dirac electrons associated with this degeneracy.

The work by Li *et al.* will stimulate further theoretical and experimental investigations to the properties of bismuth beyond the quantum limit. Elemental, yet complex, bismuth emerges, era after era, as an inexhaustible source of enigmas for condensed-matter physics.

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10.1126/science.1161381

BIOCHEMISTRY

A Natural Choice for Activating Hydrogen

The structure of a novel hydrogenase enzyme provides insights into how molecular hydrogen can be activated for use in biological processes.

Fraser A. Armstrong[1] and Juan C. Fontecilla-Camps[2]

Hydrogenases are metalloenzymes that catalyze the activation of molecular hydrogen. Microbes usually use this reaction to produce or oxidize H_2 in energy-related reactions that involve long-range electron transfer. Structures representing the two classes of "classic hydrogenases" have shown that both contain a binuclear metal center. On page 572 of this issue, Shima *et al*. (*1*) describe the structure of a third class of hydrogenase, which contains a mononuclear iron center. In this case, activation of an H_2 molecule generates a hydride that is transferred directly to an organic substrate involved in microbial methane production. The structural details revealed by the study are relevant for understanding the H_2-activation stage of the catalytic cycle of classic hydrogenases.

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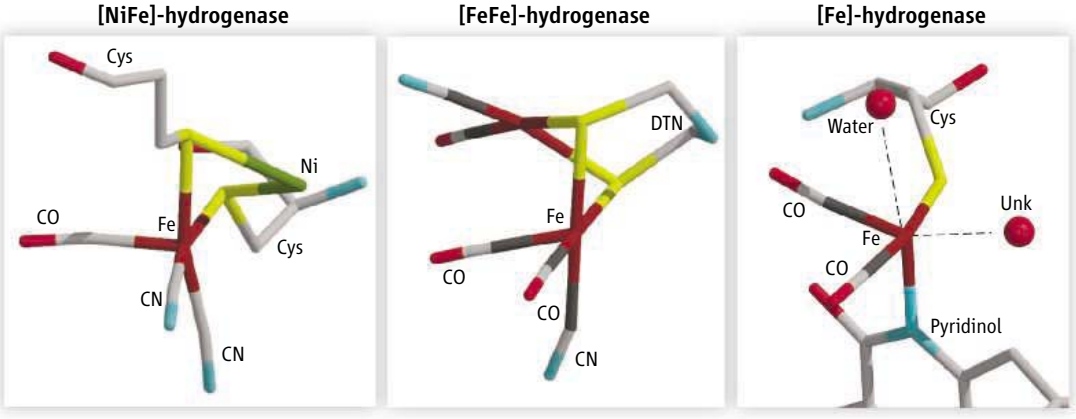
Hydrogenases contain only first-row transition metals. They are receiving considerable attention because their reactivity compares favorably with that of platinum, a precious metal. Hydrogenases are also potentially important catalysts for the large-scale industrial production of H_2 by microbial organisms. Crystal structures of two classes, now known as [FeFe]- and [NiFe]-hydrogenases, were reported several years ago; although the proteins are phylogenetically unrelated, the structures of their active sites are similar (*2*–*5*) (see the figure, left and center panels). Both contain a pair of first-row transition metal atoms, at least one of which (iron) is coordinated by the biologically unusual ligands CO and CN^- and by thiol from either the protein (cysteine) or a small organic molecule.

Both [FeFe]- and [NiFe]-hydrogenases are involved in energy processes: either the oxidation of H_2 or the formation of H_2 as a product of fermentation or photosynthesis. Unlike platinum, hydrogenases operate by a heterolytic mechanism. For H_2 oxidation, the mechanism involves prior coordination of H_2 to a metal, a reaction first discovered by Kubas (*6*). This is followed by scission of H_2 to give a metal-bound hydride and a proton that is extracted by a nearby base and removed. The hydride is then oxidized by long-range electron transfers to produce a second H^+, which is also removed rapidly. A simple reversal of these events, commencing with protonation, is likely for H_2 production.

The [Fe]-hydrogenase reported by Shima *et al*. is different, because the hydride generated by splitting H_2 is transferred directly to an electrophilic organic center in methenyl-tetrahydromethanopterin (methenyl-H_4MPT), accomplishing a key stage in the reduction of CO_2 to CH_4. This discrete hydride transfer to an organic substrate is analogous to that observed for flavin oxidoreductases and nicotinamide adenine dinucleotide phosphate–dependent oxidoreductases. Only one metal is required for catalysis, which does not seem to require any formal change in oxidation state. In the classic [FeFe]- and [NiFe]-



The active sites of the three classes of hydrogenase. The coordination centers of the equivalent iron centers are oriented to highlight their similarities. (**Left**) Fragment of the [NiFe]-hydrogenase active site (*2*). (**Center**) The binuclear [FeFe] subcluster of the [FeFe]-hydrogenase active site (*3*). DTN, di(thiomethyl)amine. (**Right**) Partial view of the active center of [Fe]-hydrogenase reported by Shima *et al.* (*1*). Unk, unknown ligand; this site appears to bind CN^-. The water molecule trans to the pyridinol ligand occupies the site thought to bind H_2.

hydrogenases, the presumed role of the second metal is to orchestrate, in part or in full, the two one-electron transfers that subsequently convert H^- to H^+. The [Fe]-hydrogenase is thus very important for our understanding of H_2 biocatalysis in general because it fulfills all the requirements for its activation and scission, the first stages of H_2 oxidation by the classic hydrogenases.

The three classes of active site share a $Fe(CO)_x(RS^-)$ unit (see the figure). The most notable feature of the [Fe]-hydrogenase (right panel) is that the coordination sphere lacks an intrinsic CN^- ligand (a stronger σ donor but weaker π acceptor than CO) and contains instead the N atom of a pyridinol ring attached to a guanyl mononucleotide. The two intrinsic CO ligands are arranged in a cis configuration. The remaining coordination sphere has some unexplained features. The site occupied by an unknown ligand is most likely the target for extrinsic CN^-. It is unlikely that this site is the one used for binding H_2 because CN^- is a noncompetitive inhibitor. In addition, a spherical electron density peak at 2.7 Å from the iron atom is modeled as a water molecule occupying the sixth coordination site. The sixth site is postulated to bind the competitive inhibitor CO and the substrate, H_2. The active site of the [Fe]-hydrogenase can be removed and transferred between related enzymes but is highly sensitive to light. This latter feature complicated its characterization substantially.

A low-spin Fe(II) site with five permanent two-electron donor ligands is well suited for binding a sixth ligand such as H_2. As described by Kubas (*6*), binding and activation of H_2 at a transition metal involves a subtle interplay between σ donation (required for binding) and back-donation (required for H-H cleavage), and it is noteworthy that the pyridinol N atom occupies the position trans to the vacant site at which H_2 probably binds. 2-Pyridinol is a versatile ligand (*7*): It can exist in two different forms with different electronic characters (an enol form with sp^2-hybridized N and a keto form with sp^3-hybridized N), can be deprotonated (increasing its donor properties), and resembles carboxylate in being potentially bidentate; indeed, it is most often encountered as a chelating ligand bridge between two metal atoms. In the [Fe]-hydrogenase, it is bound as a unidentate ligand, and the planarity of the Fe-ring system shows that N is sp^2-hybridized.

Changes in ligand status (perhaps induced by binding methenyl-H_4MPT) could play a key role in balancing σ-donor and π-acceptor characteristics at different stages of the catalytic cycle.

The remarkable convergent evolution that resulted in the presence of a common active-site $Fe(CO)_x(RS^-)$ unit in all three classes of hydrogenases deserves a comment. The π-acceptor nature of CO (and hydrogen-bonded CN^-) stabilizes low-spin, low-valence Fe with catalytic and hydride-binding properties that are normally found in second- and third-row transition metals, most notably platinum. Presumably, such metals were not available during evolution, and nature found a solution involving the much more common Fe ion and the abundant ligands CO and CN^-.

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10.1126/science.1161381

BIOCHEMISTRY

Cargo Load Reduction

Ineke Braakman and Mieko Otsu

A specific enzyme that reduces protein disulfide bonds is part of a complex that eliminates aggregated, misfolded proteins.

In eukaryotic cells, most newly synthesized secretory proteins are first translocated into the endoplasmic reticulum (ER) and transit through organelles that constitute a secretory pathway. However, a fraction of them never reach the desired native state. Instead, these proteins misfold in the ER and are retrotranslocated out of this organelle to the cytoplasm, where they are degraded by the ubiquitin-proteasome system (a process called ER-associated degradation). For efficient retrotranslocation, the disulfide bonds of misfolded proteins must be reduced, and on page 569 in this issue, Ushioda *et al.* (*1*) report that this reaction is catalyzed by ERdj5, the first dedicated reductase identified in the ER.

The most abundant ER oxidoreductase, protein disulfide isomerase (PDI), contains two thioredoxin-like domains Cys-X-X-Cys (CXXC, where X is another, but not any, amino acid), and can make (oxidize), break (reduce), and isomerize disulfide bonds, depending on reaction conditions (*2*). In principle, PDI can do the reductase job as well as ERdj5 (*3*).

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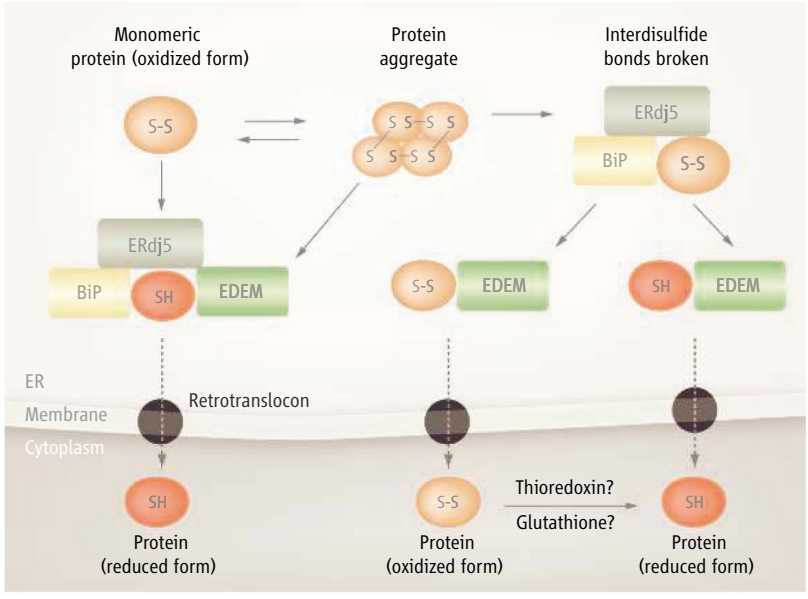
However, more than 20 additional ER-resident PDI family proteins with at least one CXXC motif have been found in mammalian cells (*4*), suggesting dedicated functionality compared to the broad activity of PDI. Ushioda *et al*. show that ERdj5 has neither oxidase nor isomerase activity in vitro, although it has four CXXC motifs (*5*, *6*). It also contains a J domain, which is found in proteins that assist heat shock protein 70 (Hsp70) in functioning as a chaperone in protein folding. This indicates that ERdj5 could function as a co-chaperone for BiP, an ER-resident protein that is a member of the Hsp70 chaperone family (*5*, *6*). Further specificity is generated by ERdj5 binding to the ER-degradation-enhancing α-mannosidase–like protein (EDEM), the lectin that receives misfolded glycoproteins from the chaperone calnexin, and directs them out of the ER to the cytoplasm (*7*, *8*). By coupling BiP and EDEM, ERdj5 may open the degradation path to misfolded proteins and aggregates (see the figure).



ER-associated degradation. Possible roles for the enzyme ERdj5 are shown. ERdj5 reduces interchain (and perhaps intrachain) disulfide bonds in misfolded and aggregated proteins in the ER, which prevents aggregation, helps dissolve aggregates, or both. ERdj5 associates with BiP and EDEM and may perform this activity as a ternary complex, directly targeting glycoproteins for retrotranslocation and degradation in the cytoplasm. Alternatively, ERdj5 and BiP first may act together on an aggregated substrate, delivering either fully reduced or oxidized monomers to the retrotranslocon. If proteins are retrotranslocated with disulfide bonds intact, both deglycosylation and reduction in the cytosol will be needed for efficient degradation.

Why is it important to reduce disulfide bonds at all when oxidized immunoglobulin kappa chain (*9*), bulky glycosylated proteins (*10*), and intact virus (simian virus 40) (*11*) can be ejected from the ER through a retrotranslocon (whose identity remains ambiguous)? It may be that instead of extending a protein chain, reduction releases proteins from thiol-mediated retention in the ER, or breaks apart protein aggregates with the combined action of BiP. Ushioda *et al*. find that ERdj5 decreases the amount of disulfide-linked aggregates of immunoglobulin J chain and dimers of α_1-antitrypsin. A disulfide-linked aggregate may be either too large to pass through retrotranslocons, or detained by a large number of chaperones, whereas individual monomeric molecules are likely optimal substrates for retrotranslocation.

Combined ERdj5 and BiP activities are not limited to glycoproteins; nonglycoproteins are substrates as well (*12*). In this case, EDEM is bypassed and BiP may directly target substrates to the retrotranslocation machinery (*9*). The combination of an oxidoreductase and chaperone in the ER is also not unique: ERp57 functions with the chaperones calnexin and calreticulin (*13*), and PDI harbors both functions. And although ERdj5 accelerates misfolded protein degradation, it is not crucial for this process because reduced expression of ERdj5 did not obliterate degradation. Any of the ER-resident PDI family proteins could function redundantly with ERdj5. On the other hand, the effects of the complete removal of ERdj5 expression are not known, and a small amount of this protein may be sufficient to stimulate degradation.

As well, reduction of disulfide bonds is not unique to protein degradation. Proper protein folding also involves constant reduction of nonnative disulfide bonds. Because ERdj5 catalyzes this process, a pool of ERdj5 should exist that is not bound to EDEM, which is underscored by its action on nonglycoprotein substrates.

A reductase must be kept in a reduced form and therefore needs an electron donor. In vitro, dithiothreitol or glutathione can fulfill this role, but in cells, electrons must be provided inside the ER. Analogy with the bacterial periplasmic space (where electron transport processes occur) suggests a role for cytosolic thioredoxin, whereas oxidases donate their electrons to proteins in an independent electron transport cascade. However, cytosolic electron donation requires a transmembrane domain–containing protein for this transport step. Instead, Ushioda *et al.* suggest ER-localized nicotinamide adenine dinucleotide phosphate as an alternative electron donor. A theme among oxidoreductases is the cooperation of two active sites within one protein, and the four active sites of ERdj5 in principle lend themselves to such a mechanism.

Although proteins with major roles in ER-associated degradation have been characterized, and several proteins have been put forward as the retrotranslocon—Sec61 (*14*), derlins (*15*–*17*), and lipid droplets (*18*)—it is fair to say that the retrotranslocation mechanism remains a black box. How proteins cross the ER membrane to the cytosol still is an open question. Another major question is why there are so many oxidoreductases in mammalian cells, given that PDI is sufficient to carry out all disulfide-reducing activity and can act on most substrates tested. Answers will likely come from studies that address tissue and substrate specificity, and—as exemplified by ERdj5's association with BiP and EDEM—from partnerships that direct activity toward a specific process.

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10.1126/science.1162125

PHYSICS

Let Quantum Mechanics Improve Your Images

Entanglement of light beams allows images to be transmitted and recorded in ways that surpass classical limits.

Robert W. Boyd

Images are superb conveyors of information. Recent research has shown how subtle quantum mechanical aspects of light can profoundly influence the nature of image formation.In this issue, two important advances in this emerging area of quantum imaging are presented. Wagner *et al.* (page 541) (*1*) report on the behavior of two beams of light that are quantum mechanically entangled in position and direction of propagation—that is, the outcome of measurements on one beam depends on what sort of measurements have been performed on the other beam. Boyer *et al.* (page 544) (*2*) show that two image-bearing light beams can be entangled such that strong quantum correlations exist both between the two beams and between individual image features within each beam. They find two sorts of quantum correlations: The intensities of the two beams fluctuate in unison, at a level not permitted by classical statistics, and the noise in one part of the light field can be reduced, or "squeezed," at the expense of another part.

The formation and manipulation of optical images is often treated classically, even though the light fields that carry images are quantum mechanical in nature. One well-known consequence of the quantum nature of light is the discreteness of the energy distribution within an image. Light arrives at each image point in the form of an integer number of photons. When light levels are low, the quantum nature of the light field thus leads to a grainy image (*3*), no matter how perfect the image-recording medium might be.

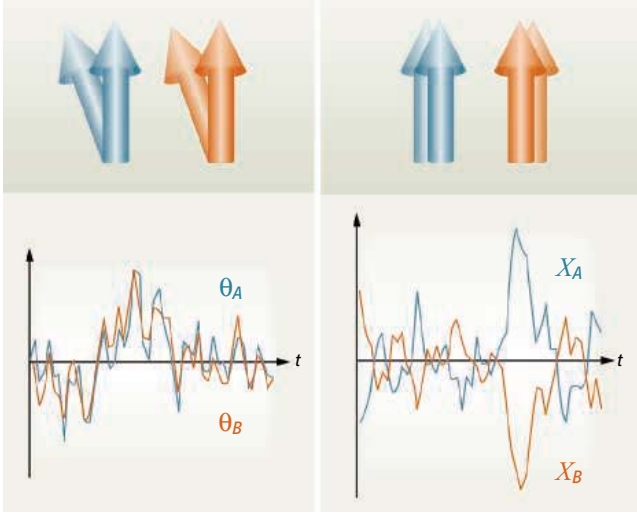
The field of quantum imaging (*4*, *5*) attempts to exploit these quantum effects to form images with finer detail or better sensitivity than those available with classical techniques. Examples of current research include the use of entangled photons to perform interferometric lithography with resolution exceeding the Rayleigh limit (*6*) and the possibility of performing interaction-free (*7*) and coincidence (*8*, *9*) imaging.

Wagner *et al.* present intriguing new results on the properties of entangled light beams. The authors first point out that the position X of a light beam (its geometric center) and its direction of propagation θ must obey a Heisenberg uncertainly relation: Either X or θ can be measured to high accuracy, but they cannot both be measured simultaneously. In the context of classical physics, this result is simply a statement that diffraction effects will cause a light beam of finite diameter D to diverge into a cone angle of approximately λ/D, where λ is the wavelength of the light—that is, there are limits to focusing a light beam based solely on its wavelength.

However, the situation is much richer when viewed quantum mechanically, and some of these limits can be beaten up to a point. Two separate light beams can be constructed so that they are entangled—the properties of either beam cannot be described independently, so one can speak meaningfully only of the combined properties of the total system.

Using some sophisticated tricks developed during the past two decades, Wagner *et al.* produce two light beams, A and B, that are entangled in that the propagation directions of the two beams are correlated—their directions fluctuate in unison. Thus, the variance of the difference in propagation directions, which we designate as $V(\theta_A - \theta_B)$, is much smaller than the variance of either of the individual propagation directions (see the figure). Simultaneously, the beam positions are anticorrelated in the sense that $V(X_A + X_B)$ is much smaller than the variance of the position of either beam. In classical physics, either of these conditions can occur, but it is not possible to achieve both simultaneously.

If the system had complete quantum correlation, $V(\theta_A - \theta_B)$ and $V(X_A + X_B)$ would both vanish. However, imperfections in the laboratory setup prevent the correlations from being complete. Wagner *et al.* apply two of the standard criteria for demonstrating quantum correlation and find that their data meet each of them. One is inseparability—the two-beam wave function cannot be factorized into the product of individual wave functions (*10*). The second is the Einstein-Podolsky-Rosen (EPR) limit (*11*). This condition states that the system displays an EPR paradox, in that the collapse of the two-particle wave function occurs instantaneously (the aspect that troubled Einstein). EPR entanglement between two individual photons has been observed previously (*12*), but this experiment demonstrates entanglement between entire beams of light.



Entangled light beams. Entanglement is illustrated between two laser beams (A in blue and B in red). (**Left**) The direction of propagation fluctuates, but the fluctuations are highly correlated, so that the directions change in unison. (**Right**) Two beams whose positions are anticorrelated—as one beam moves left, the other moves to the right. For either beam studied individually, the position fluctuations ΔX and direction fluctuation $\Delta\theta$ are related by diffraction laws and obey an uncertainly relation $\Delta X\, \Delta\theta > \lambda/4\pi$, where λ is the wavelength of light. However, because of the correlations between the two beams, the uncertainty product of the differences $[\Delta(X_A + X_B)][\Delta(\theta_A - \theta_B)]$ can be much smaller than $\lambda/4\pi$

In a second advance in quantum imaging, Boyer *et al.* describe two related laboratory experiments that investigate the properties of entangled image-carrying beams. In the first

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experiment, the authors impress an image onto a probe beam by passing it through an amplitude mask. They then pass this beam through an optical amplifier with a gain of ≈4.5 that operates by means of four-wave mixing in an atomic rubidium vapor. The four-wave mixing process converts two pump photons into signal and conjugate photons. Thus, in addition to amplifying the probe beam, this process generates a conjugate beam that is the twin of the probe beam. This conjugate beam mimics the properties of the probe beam, even to the extent of replicating its quantum fluctuations.

To demonstrate this behavior, the authors measured the intensities of the two beams and the difference in their intensities. Fluctuations in the intensity difference are smaller by a factor of 3.5 than are the quantum fluctuations in either individual beam. This strong quantum correlation exists not only for the entire beam but also for the individual parts of the image carried by the beam.

Their second experiment explores some of the more subtle aspects of the quantum correlations between two entangled beams. For this experiment, no probe beam is injected into the optical amplifier. Only the zero-point fluctuations associated with the electromagnetic "vacuum" that occur spontaneously are allowed to seed the amplification process. In this case, the two output beams are in the form of pure noise fields, but the noise properties are strongly correlated and can be studied by measuring each of the beams with homodyne detection. These measurements show that certain linear combinations of the two beams possess strong quadrature squeezing (for example, the noise of the part of the field that oscillates as cos ωt can be very much smaller than that of the part that oscillates as sin ωt.)

Remarkably, this squeezing persists even if an arbitrary image is impressed on each of the local oscillators used in the homodyne detection process. By impressing an image onto the local oscillator's field, the homodyne detection process sees only that part of the noise field that possesses the same spatial structure as the local oscillator field. This procedure demonstrates that strong quantum correlations exist between the probe and conjugate fields, even when they are projected onto nearly arbitrary spatial modes.

The work of Wagner *et al*. and Boyer *et al*. illustrates the richness of the phenomena that can occur when quantum effects are studied in the context of image formation. Such quantum effects may find use in enhancing the process of image formation. These studies complement recent progress in using the transverse nature of the light field (*13*) to increase the information it carries per unit time, which is of interest in the related field of quantum information science.

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10.1126/science.1161439

MATERIALS SCIENCE

Bulk Metallic Glasses

Improved testing and a better understanding of the properties of bulk metallic glasses will lead to new avenues for commercial use.

Cormac J. Byrne and Morten Eldrup

Metals and alloys generally exist as crystalline materials. Around 1960, it was discovered that rapid cooling of the melt resulted in the formation of alloys in glassy states. About 30 years later, multicomponent alloys were discovered that could be solidified as glasses at much lower cooling rates (~1 to 100 K/s or lower), resulting in samples larger than ~1 mm. Compared to their crystalline counterparts, these bulk metallic glasses (BMGs) often exhibit high compressive strength, good corrosion resistance, and large elasticity (*1*). Despite the identification of a vast range of BMGs and applications including sporting goods and electronic casings, the development of methods for rationally designing BMG alloys is still an important task.

BMGs with the best glass-forming abilities are Zr- and Pd-based, often reported in terms of a "critical casting diameter." Critical casting diameters up to 70 mm have been reported in Pd alloys with cooling rates lower than 10^{-1} K/s (*2*). In many systems, microalloying has been found to improve the glass-forming ability (*3*). For example, aided in part by microalloying, BMG steels have generated interest because of their strength and cheaper constituent materials than the traditional Pt, Pd, and Zr classes of BMGs (*4*, *5*).

Small changes in composition can lead to large changes in properties. Several empirical rules exist correlating a single physical parameter with glass-forming ability, but only some of these correlations are useful for predicting universal glass-forming ability (*6*). These rules still require knowledge of properties of the alloy to allow prediction of glass-forming ability. Models that only require information about the constituent elements as inputs remain a challenge.

A better understanding of the atomic structure of BMGs is developing, primarily through analyses of the simplest binary alloys. The popular portrait of the BMG structure is a randomly packed assembly of the different atoms. However, although BMGs lack long-range atomic order, they do exhibit short- and medium-range order over several atomic lengths (*7*). The identification of polyamorphism (different amorphous phases) indicates some of the inherent complexities involved in any atomistic-scale investigation of multicomponent BMGs (*8*).

Compared to other engineering materials, BMGs are used in small volumes. Most are quite strong in compression, and several optimized alloy compositions show notable compressive ductility (*9*, *10*). Many engineered

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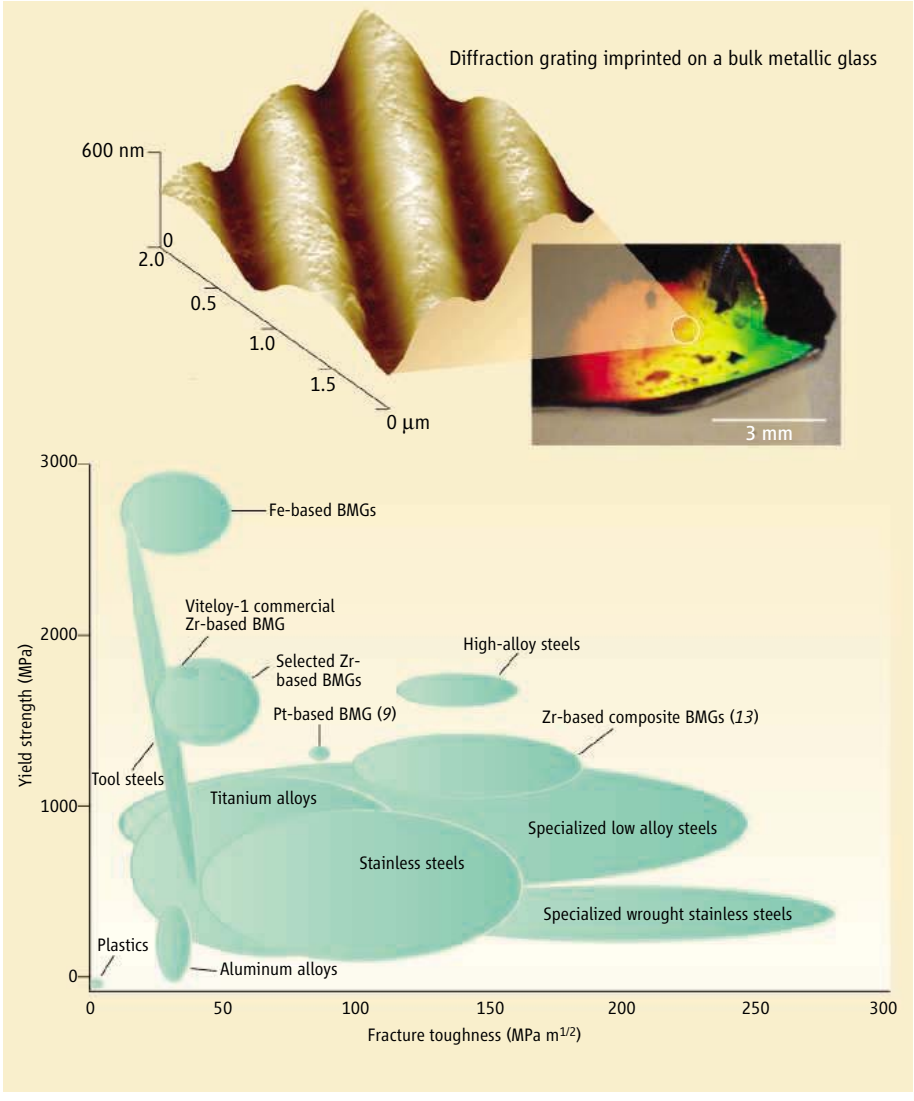

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How BMGs measure up. (**Bottom**) A comparison of the strength and fracture toughness of a few selected BMGs with those of some crystalline metals and plastics. BMG properties are becoming increasingly competitive compared with those of other engineering materials. (**Top**) A sine diffraction grating prepared by pressing a Ni die into a MgCuY bulk metallic glass above its glass transition temperature (~165°C). The nanometer-scale replication is small enough to allow light to be diffracted.

parts require good toughness and strength in tension rather than compression, requirements that most monolithic BMGs cannot meet. Ductility has been shown to increase dramatically at small length scales; hence, microscale and smaller components are alluring (*11*). On a larger scale, recent reports of "designed" BMGs indicate substantial improvements in fracture toughness (*12*). In parallel, BMG composites (amorphous matrix with a crystalline second phase) have been well designed by matching the microstructural length scale of the crystalline phase to crack length scales, resulting in high tensile ductility and fracture toughness, thus making the composites very competitive with crystalline metals (*13*).

Production technologies for crystalline metals are mature, whereas those for BMGs are in their infancy. A variety of casting techniques and fluxing strategies are now used to produce BMGs. There is some evidence that BMG properties are dependent on cooling rate, so the choice of manufacturing route can be important, particularly for net-shape casting (*14*). Gas atomization or mechanical alloying can be used to produce amorphous powders, which can subsequently be compacted and consolidated into a BMG. Porous metallic glasses (e.g., foams) have been made via a variety of techniques to introduce ductility and manipulate the density (*15*).

A unique property of BMGs is their ability to reversibly transform from the low-temperature glassy state into a supercooled liquid state above a glass transition temperature. Many BMGs are not well suited to conventional forming/machining at room temperature, but are readily processable in the supercooled liquid region (SLR), where the viscosity of the metal can drop by several orders of magnitude. Less force is thus required to get the material to flow during forming processes. Forming in the SLR has been successful on most systems, but with Pt- and Pd-based glasses in particular. Extrusion, closed-die forming of microcomponents, and many more innovative techniques such as blow molding (*16*) have also been demonstrated. Maximizing the extent of the SLR can yield lower viscosities, thus requiring lower forming forces. A promising development was a recent report of Zr-based alloys with exceptionally large SLRs (*12*).

BMGs can be formed into very precise shapes while in the SLR, with surface features on the order of nanometers obtainable (*17*) (see the figure). Often the quality with which the die can be manufactured becomes the limiting factor in these nanoforming operations. In this regard, BMGs themselves can be used to form high-precision dies for forming other BMGs or plastics.

BMGs are a class of materials that offer many desirable and unique processing routes and properties. To exploit these possibilities fully, standardized testing and reporting of properties are necessary, so that databases of BMGs and their properties can be reliably compiled and used. An improving universal description of the structure and behavior of BMGs should lead to better predictive tools for their design and thus a greater propensity for commercialization.

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10.1126/science.1158864

ENVIRONMENT

Pacific Division Meeting Probes Threats to Hawaiian Paradise

WAIMEA, Hawaii—From their research station high on the forested slopes of Mauna Kea, Rebecca Cann and Leonard Freed have watched with alarm in recent years as a beautiful orange bird called the akepa has plunged toward extinction in their study site. They have seen females begging for food until their voices faded to silence, and young and old birds die en masse.

Endangered: *Achatinella sowerbyana*

At the recent annual meeting of the AAAS Pacific Division, researchers described the collapse of the akepa as just one of many ominous signs for the Hawaiian paradise. Native birds are threatened by introduced predators and diseases. Land snails with exquisite shells have suffered from deforestation and shell-collectors. Sea turtles are injured and killed by fishing crews. Brackish coastal ponds that are home to rare flora and fauna have been undermined by an invasion of nonnative fish.

"The serious problems of environmental stress and degradation are not unique to Hawaii—they're being felt to varying degrees on most islands and many coastal areas in the Pacific Basin," said the division's executive director, Roger Christianson, a biologist at Southern Oregon University. "The meeting provided researchers and educators across a wide array of disciplines an important forum in which to discuss their efforts to study and, hopefully, protect these treasures before they disappear."

The Pacific Division's 89th annual meeting brought more than 300 researchers, educators, students and others to the Island of Hawai'i—the Big Island—from 15 to 20 June for serious scientific discussion and a celebration of Pacific Island culture. More than two dozen symposia, lectures, and workshops ranged across fields and included sessions on nanotechnology, science education, neuroscience research into health problems shared by indigenous people in Hawaii and Alaska, and U.S.–Asia scientific cooperation.

But the recurring theme of the meeting was the profound environmental challenges confronting the Pacific islands, many of which were protected by isolation until humans arrived.

At a half-day symposium, top malacologists described how humans and nonnative predators—including rats, pigs, and voracious meat-eating snails—have decimated native land snails that are important cultural figures for indigenous Hawaiians. Already, as many as 80% of the species are gone.

"You knock out one species, and you knock out another species, and you knock out another species—at some point, something is going to happen," said Michael G. Hadfield, a professor of zoology at the University of Hawaii at Manoa. "There is going to be a shift, and that ecosystem is not going to be what it was before….The snails are the canary in the coal mine—they're telling you that something isn't right."

The family of Hawaiian honeycreepers, including the akepa, has followed a similar arc: They evolved to broad and colorful diversity, but many are now extinct or endangered.

The akepa had held its own between 1987 and 1999 in the presence of Japanese white-eye, an insect-eating bird introduced to Hawaii in 1929. But beginning in 2000, the husband-and-wife team of Freed and Cann noted a dramatic increase in the number of white-eye, and as that bird increased, it competed with the akepa and other native species for food. During 2006, the akepa population collapsed. The endangered akiapolaau and Hawaii creeper also declined.

Between 2000 and 2005, Cann, Freed, and colleagues came to a startling realization: "Young females had all but disappeared in the population." They determined that early-nesting females normally produce sons, the larger sex. "Food limitation has now become more severe over the season," Cann said, "so only early-nesting females were breeding successfully, with adverse consequences for the population."

Facing long odds, researchers now are working to save native snails and birds. Hadfield's colleagues are studying snail DNA and breeding them in their laboratories. While the akepa is being bred in captivity at a Hawaiian center affiliated with the San Diego Zoo, Freed is urging U.S. wildlife authorities to control white-eyes to protect native birds.

In a plenary address, outgoing Division President Terrence Gosliner described how

INTERNATIONAL

AAAS Science Literacy Text Translated into Japanese

When a group of Japanese scientists and educators sought a way to boost the scientific literacy of their nation's citizens, they were inspired by AAAS's *Science for All Americans*, using it as a guide "to show the essence of science to be shared by all people," said Kazuo Kitahara, a theoretical physicist and director of Japanese science literacy initiatives.

Just 3 months after its release, the Japanese translation of the seminal science literacy text already has been downloaded more than 3000 times from the Web site of AAAS's Project 2061, demonstrating that the education concepts in the book have global applications.

Science for All Americans defines what every high school graduate should know and be able to do in science, mathematics, and technology in order to understand and thrive in the modern world. The new translation was funded by the Japanese government's Ministry of Education.

Science for All Americans served as a basis for Project 2061's *Benchmarks for Science Literacy,* a curriculum-design tool for educators. Both also have been translated into Spanish and Chinese. "I'm delighted that so many countries continue to find *Science for All Americans* useful to their efforts to improve science education," said Jo Ellen Roseman, director of Project 2061.

Science for All Americans was first published in 1989 by Project 2061, AAAS's long-term initiative to reform science, math, and technology education at the K–12 levels. Free online copies of the text and its translations are available at www.project2061.org/publications/sfaa.

—Molly McElroy

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The native Hawaii akepa (left) is declining because of competition for food from the nonnative Japanese white-eye (right).

CREDITS: HAKALAU FOREST BIOLOGICAL FIELD STATION INTERNS

local museums could play a crucial role in Pacific Basin conservation efforts. Gosliner is the senior curator of invertebrate zoology at the California Academy of Sciences in San Francisco, and as the Academy prepares to open its state-of-the-art "green" building in September, he and his colleagues have built a Philippine coral reef exhibit in a 210,000-gallon tank.

They have collected corals sustainably, without harming the lush Philippine reefs. They have helped develop aquaculture techniques that can be used in the commercial coral trade. And, he said, the project has helped create important partnerships with conservation groups in the Philippines and the Bay Area's large Filipino-American community.

The four regional divisions of AAAS—Pacific, Southwestern and Rocky Mountain, Arctic, and Caribbean—serve as regional networks for scientists, engineers, and other members. The Pacific Division, founded in 1914, includes 30,000 AAAS members.

ETHICS

Experts Urge Action Against Gene Doping

Scientists, governments, and sports officials should collaborate on dramatic new efforts to prevent the "imminent" use of illicit gene transfer by athletes and their medical advisers, experts said at a world conference cosponsored by AAAS.

The World Anti-Doping Agency meeting in St. Petersburg, Russia, urged scientists to document natural genetic differences in physical traits so that future tests could assess whether an athlete's genes had been modified. Governments should develop sanctions for doctors and other licensed professionals who attempt illegal gene transfer in athletes, the group concluded. And, it said, governments should impose stronger regulation on commercial genetic technologies that are already becoming available over the Internet.

Gene doping is "feasible, inevitable, and imminent, something that will happen in years rather than decades," said Dr. Theodore Friedmann, former president of American Society of Gene Therapy and current director of the University of California–San Diego Gene Therapy Program.

Mark S. Frankel, director of the AAAS Scientific Freedom, Responsibility and Law Program, said gene doping may become a factor as early as the 2012 Olympic Games. "The feeling is that in that amount of time, the science can make significant inroads," said Frankel, one of the conference organizers. "In the competitive world of sports, everyone is looking for an edge, and there's every reason to believe that coaches and their athletes, maybe even their agents, will be looking at this."

The declaration by the World Anti-Doping Agency (WADA) was released this month after more than 60 representatives from 16 countries met for its Third Gene Doping Symposium from 10 to 11 June. In the past, WADA has fought athletes' use of steroids, blood doping, and other artificial means of improving performance.

Gene doping would add new genes or manipulate an athlete's own genes that control muscle growth and development or endurance, for instance. New genes could be added to cells and tissues using a targeted virus or other delivery method, but researchers are also preparing for the possibility that an athlete's own genes could be modified by treatment with genetic elements or even drugs. These processes would exploit transfer techniques developed by researchers for therapeutic purposes, such as restoring immune function in certain genetic diseases.

Although there have been no documented cases of gene doping, the techniques used in legitimate gene therapy have grown more effective at the same time that the global marketplace has made the techniques more widely known and accessible. Together, these factors point to an urgent need for concrete enforcement and regulation measures aimed specifically at gene doping, along with a push to develop viable screening tests for illicit gene transfers, experts said at the symposium.

Friedmann predicted that athletes may be the pioneers in a new world of human enhancement, where methods of genetic manipulation are used to alter a person's abilities or physical appearance rather than treat disease.

"Science has moved so quickly in gene therapy and because it moves so quickly, it makes the nontherapeutic use of these kinds of methods much more likely," he noted. "And the sooner it pops up in sport, the more likely it is to pop up in other areas."

Friedmann believes that it's important to draw the legal and ethical lines now between gene therapy and gene doping among athletes, so that the public will understand better the risks and limitations of the science. At the moment, participants in gene therapy studies are protected by the oversight of institutional review boards, governmental agencies, and international codes of human experimentation such as the Helsinki Declaration. If athletes—and those who follow them into the new world of genetic enhancement—receive illicit gene transfers without these protections, "such manipulations would clearly be unethical," Friedmann said.

WADA was established in 1999, an outgrowth of efforts by the International Olympic Committee to combat doping in sports. Its work is supported by governments, intergovernmental organizations, and other public and private bodies.

"I think it's extremely important for WADA as a world organization to have feedback from a scientific organization like AAAS, bringing its voice and its reflections on some of the issues we are facing beyond the technical aspects," said Olivier Rabin, the agency's science director.

The Scientific Freedom, Responsibility and Law Program released a report on human enhancement in 2006 and more recently helped organize a session on human enhancement at the 2008 AAAS Forum on Science and Technology Policy in Washington, D.C.

The risks associated with fledgling genetic technologies—such as the cases of leukemia that developed in children treated with gene therapy for x-linked severe combined immunodeficiency, or "bubble-boy disease"—are well known among researchers. WADA supports an extensive education program to warn the sports community about these potential dangers, but Frankel said he was surprised to hear how often researchers are contacted by coaches who volunteer their athletes as test subjects for gene-transfer experiments.

—Becky Ham

Studying Atomic Structures by Aberration-Corrected Transmission Electron Microscopy

Knut W. Urban

Seventy-five years after its invention, transmission electron microscopy has taken a great step forward with the introduction of aberration-corrected electron optics. An entirely new generation of instruments enables studies in condensed-matter physics and materials science to be performed at atomic-scale resolution. These new possibilities are meeting the growing demand of nanosciences and nanotechnology for the atomic-scale characterization of materials, nanosynthesized products and devices, and the validation of expected functions. Equipped with electron-energy filters and electron-energy–loss spectrometers, the new instruments allow studies not only of structure but also of elemental composition and chemical bonding. The energy resolution is about 100 milli–electron volts, and the accuracy of spatial measurements has reached a few picometers. However, understanding the results is generally not straightforward and only possible with extensive quantum-mechanical computer calculations.

To see the atomic structure of matter is an old dream in science that has now become a burning issue with the advent of nanoscience and nanotechnology, for which atomic-scale synthesis requires atomic-resolution characterization of the results (*1*). In the past two decades, substantial progress has been made in electron microscopy (EM) to achieve this objective, and in a limited number of favorable cases, it has been possible to perform structure investigations at atomic-scale resolution (*2*). However, only the successful construction of aberration-corrected electron lenses in the 1990s (*3*, *4*) set atomic-resolution EM on its path to becoming a more generally applied technique in advanced condensed-matter physics and materials science (*5*–*7*).

Over and above the high-precision mapping of atom positions, aberration-corrected EM enables the occupancies of atom sites to be determined and allows atomic-scale imaging of chemical composition and bonding by combining high resolution in energy and space with the use of spatially resolved electron-energy–loss spectroscopy (EELS) to map particular electron states localized at or between atoms. These features explain the large number of orders worldwide for the new generation of aberration-corrected electron microscopes that have come onto the market since around 2004. On the other hand, work with aberration-corrected optics also shows how demanding the new techniques are and that they are as yet by no means routine. Furthermore, because atomic-scale resolution means probing the quantum world, contrary to both intuition and frequently held views, understanding what the "images" show is generally not straightforward. As a consequence, in many cases, the ultimate result of state-of-the-art microscopy is an atomic model on a computer rather than an image.

Scientists employ transmission EM (TEM) in two basically different technical variants (*8*). In conventional TEM, the specimen is illuminated by a near-parallel bundle of electrons, and the image is formed by a sequence of lenses equivalent, in principle, to those used in a light-optical microscope. In scanning TEM (STEM), a fine probe is formed by optically focusing the incident electrons and is then scanned across the specimen. The transmitted electrons are registered by detectors, and the resulting signal is displayed on a video screen. In conventional TEM, the aberrations of the objective lens are decisive for an image's quality, whereas in STEM, the aberrations of the probe-forming lens determine the quality. In principle, two types of aberrations are always involved: geometrical aberrations, such as spherical aberration, and chromatic aberrations, which arise from the electron-energy dependence of the refraction properties of magnetic fields. Current lens designs can only correct for the former. To reduce the effect of chromatic aberrations, field-emission electron sources and, in some cases, energy filters (monochromators) are used in the electron-beam–forming system of the new generation of 100-to-300-keV instruments.

An ideal converging lens would image a point in the object to a corresponding point in the image. In reality, as a result of aberrations, the image is broadened into a point-spread disk. This is illustrated schematically in Fig. 1A for the case of a spherical aberration. Point spread arises from the refraction power of a real lens increasing with the angle that the beams entering the lens make with its optical axis. As a result, the electrons that scattered in the specimen at high angles come to a focus some distance in front of the Gaussian image plane that is defined by the low-angle beams. The focal length of an electromagnetic lens can be easily varied by adjusting the current through the lens coils. Such defocusing also induces point spread and is treated as an aberration. Although these two represent the most substantial aberrations, there are many more that contribute not only to a broadening but also to an angular distortion of the point-spread disk (*6*, *9*, *10*).

Progress has been achieved in recent years because systems that are largely free of aberrations can be constructed with aberration compensation brought about by adding to a lens optical elements that exhibit the same aberrations as

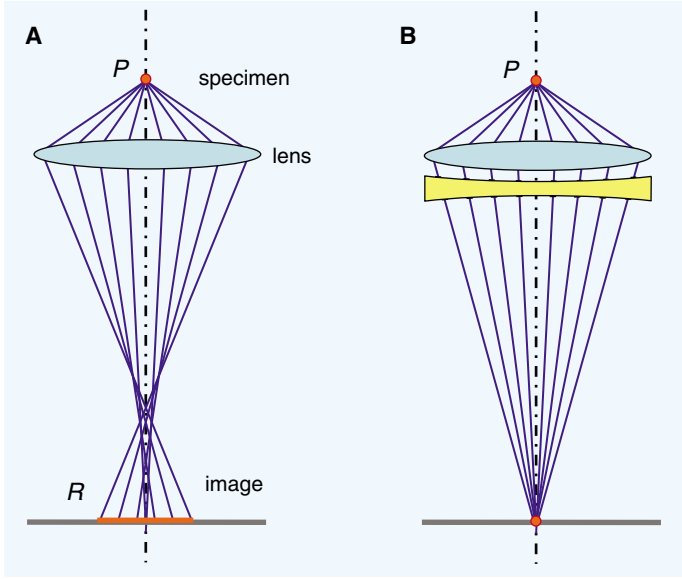

Fig. 1. (**A**) Schematic illustration of spherical aberration of a converging lens. The scattering power increases with the increasing angle (with respect to the optical axis) at which the electrons enter the lens. As a result, the focal length of the beams passing the lens at its periphery come to a focus a distance in front of the Gaussian image plane that is defined by the paraxial, low-angle beams. The image of a point *P* of the specimen is broadened into a "point-spread disc" of radius *R*. (**B**) Spherical aberration is compensated by combining the converging lens with a suitable diverging lens. In electron optics, diverging lenses are realized by combinations of multipole lenses.

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the lens but of the opposite sign. In particular, the spherical aberration of a converging lens is corrected by adding a diverging lens that compensates for the too-high refraction power of high-angle scattered beams (Fig. 1B). This is achieved by a double-hexapole system (*11*, *12*) in conventional TEM and in STEM. For the latter, multiple quadrupole-octopole systems (*10*, *13*) are also used.

Conventional TEM

The basis for studying atomic structures by TEM is the quantum mechanical interaction of the incident electron wave field with the atomic potential. It is this interaction that supplies information about the specimen's interior structure. This information is contained in the electron wave function at the exit plane of the specimen. It is this wave function that is representing the object for the subsequent optics. Although individual single atoms deposited on a transparent substrate can be imaged by using TEM, the bulk of the work concerns crystalline structures. Because of the limited resolution of the optical system, these are usually oriented in such a way that a major crystallographic axis is adjusted to be parallel to the direction of the incident electron beam. Thus, the projected lateral atom distances are adopting maximum values, and the atom columns extending along the viewing direction are imaged end-on.

The exit-plane wave function cannot be derived in a straightforward way from the intensity distribution in the image. This is because atomic structures are phase objects; because phases (equivalent to Zernike phase-contrast microscopy in light optics) cannot be "seen," contrast has to be created by deliberately introducing particular phase shifts into the electron wave field by suitably defocusing the objective lens. This is the basis of the classical Scherzer technique of phase-contrast TEM (*14*). However, the defocus-induced phase shifts are highly nonlinear in angle and affect the image intensity distribution in a very complex manner; thus, there is no direct way to derive the desired exit-plane wave function.

A solution to this problem is provided by computer-based wave-function retrieval techniques. There are a number of variants, but all have in common that the microscope is operated as an interferometer, which exploits the fact that the objective lens introduces phase shifts into the electron waves. The technique most widely used is the focus-variation technique (*15*, *16*). A series of typically 20 images are recorded by a charge-coupled device camera while the objective-lens focus is varied incrementally step by step. These images are transferred, pixel by pixel, into a computer, where the exit-plane wave function is calculated via least-squares fitting to a simulated image series (*17*). Besides defocus, all other lens aberrations also affect the image intensity distribution, which requires the handling of typically more than 10 parameters. These values must either be known from dedicated scattering experiments (*9*) or compensated to negligible values before taking the image series.

In spite of some progress (*18*, *19*), no technique is known that allows us to calculate the potential and the underlying structure "backwards" from the exit-plane wave function. The solution is to do a "forward" calculation, in which a Schrödinger form (with relativistically corrected electron mass and wavelength) of the Dirac equation in small-angle approximation is solved numerically for a model structure based on a first guess and iteratively improved to obtain a best fit between the calculated and experimental exit-plane wave function. In addition to the formidable task of properly adjusting in the model the positions of a large number of atoms with atomic precision, this procedure is hampered by the fact that in atomic dimensions there is no direct access to such important imaging parameters as sample thickness and the precise direction of the incident electrons. There is no other solution but to treat these parameters as variables that have to be determined in the fitting procedure. The result generally is therefore not an image in the conventional sense but a computer model of the structure that gives the atomic species and coordinates. These procedures are facilitated if the structure is known in some areas of the imaged sample region because this information can be used as a reference. This is the case if defects in otherwise perfect structures are investigated. Also, the presence of various types of atoms with major differences in atomic scattering power accelerates the computer fit. In very thin samples, computer modeling benefits from the projected-potential approximation, according to which the maxima in maps of the phase part of the complex electron exit-plane wave function are considered as a projection of the atomic-potential minima in the sample (*8*).

The new contrast theory for aberration-corrected instruments shows that optimum contrast at a minimum point spread can be achieved by combining a small defocus with a small negative value of the spherical-aberration parameter C_S, produced by overcompensation by a few percent of the value in the uncorrected instrument (*20*). Under these negative spherical-aberration imaging (NCSI) conditions, atoms appear bright on a dark background; the contrast is so strong that oxygen and other light atoms, such as nitrogen or even boron, can be seen in the original images (*21*). This is substantial progress as compared with conventional TEM, in which oxygen could only be seen in the reconstructed exit-plane wave function (*15*, *22*). Besides the reduction in point spread, which is equivalent to higher resolution, the dramatic increase in contrast achieved by NCSI is considered to be one of the major advantages of spherical-aberration correction (*23*, *24*).

An example for NCSI is given in Fig. 2, showing a Σ3{111} twin domain boundary in $BaTiO_3$. With reference to the schematic of the structure, all the atom species, including oxygen (arrows), can be identified in the image. By quantitatively evaluating the local intensity signal, the occupancy (the fraction of sites occupied in a given atomic column seen end-on) can be measured. As depicted in the upper part of the figure, the occupancies thus obtained amount to only 40 to 70% of the bulk value far away from the defect (*25*). This provides direct evidence of oxygen deficiency at the Σ3{111} boundaries in $BaTiO_3$. The corresponding change of the electronic states provides an explanation for the detrimental influence of this type of defect on the electronic properties. Such measurements are not possible in uncorrected instruments because the intensity measured at a given atomic site is falsified by the effect of aberration-induced point spread, leading to parasitic or "cross-talk" intensity at this site originating from neighboring atoms.

The described quantitative techniques have also been applied successfully to investigate lattice defects, stacking faults, and dislocation cores in GaAs and InGaAs/GaAs-based

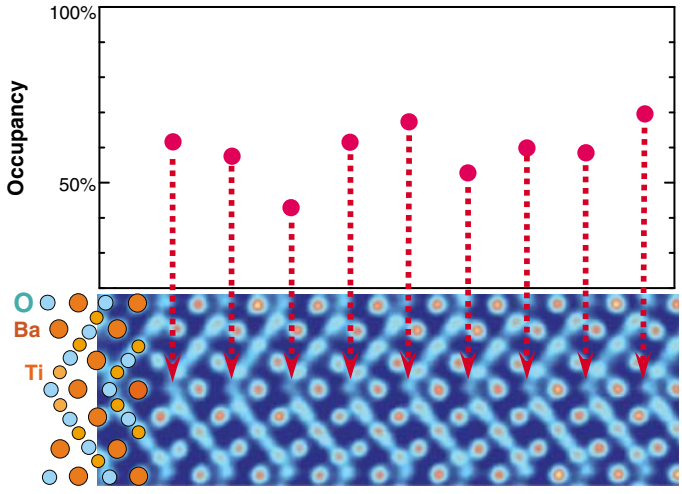

Fig. 2. Σ3{111} twin boundary in $BaTiO_3$. All atomic species, including oxygen, can be identified. The atomic resolution is confirmed by image simulations. These indicate that, because of negligible point spread, neighboring atoms have no effect on the intensity measured in a given atomic position. This provides the basis for the quantitative site-occupation measurements. The local intensity values indicate that, in the individual oxygen-atom columns seen end-on, only between about 40 and 70% of the sites are occupied. This provides evidence for oxygen deficiency, which is presumed to have a detrimental influence on electronic properties [adapted from (*25*)].

heterostructures at atomic resolution (*26*). An investigation into the surfaces of nanometer-sized platinum catalyst particles provided clear evidence that the outermost layers consist of irregular islands of atoms favorable to the dissociation of H_2 and O_2 molecules (fig. S1) (*27*).

Resolution and Accuracy in Conventional TEM

The resolution of an electron microscope is generally specified on the basis of a Rayleigh-type criterion, that is, by the distance at which two atomic contrast maxima can be separated in the image (*8*). For the current generation of aberration-corrected 300-keV instruments, this value is about 80 pm (0.08 nm). The accuracy at which the separation of well-isolated atoms can be measured is, however, better by more than an order of magnitude. In a study of 90°-tilt grain boundaries in $YBa_2Cu_3O_7$ (fig. S2), atom relaxations as small as 6 pm were measured, and an accuracy below 5 pm was demonstrated using an aberration-corrected instrument with a Rayleigh resolution of 120 pm (*28*).

This precision of aberration-corrected conventional TEM allows values for physical parameters to be derived directly on the atomic scale. This was demonstrated by an investigation into the structure of polarization domain walls (Fig. 3A) in the ferroelectric Pb$(Zr_{0.2}Ti_{0.8})O_3$ (PZT) (*29*). Ferroelectricity arises from characteristic shifts of atoms on the unit-cell scale, which induce charge-dipole formation by lowering the local symmetry. The picometer-precision measurement of the shifts of individual atoms, including oxygen (Fig. 3B), allowed the derivation, unit cell by unit cell, of the local strength and direction of the polarization vector.

STEM

As in conventional TEM, access to the specimen structure is provided in STEM by the quantum mechanical interaction of the incident electron wave field with the atomic potential. However, there are two major differences that are decisive for image formation in STEM. The first arises from the geometry of the fine probe rastered across the specimen. It is formed as the tip of a cone produced by a convergent bundle of electrons. The associated angular range of the incident electrons gives rise to characteristic electron states inside the sample and to a corresponding electron exit-plane wave function. The second characteristic of STEM arises from the detector geometry beneath the specimen. Depending on the radial position of the detector, STEM offers two different imaging modes. Bright-field imaging is based on the low-angle scattered electrons, yielding images subject to phase contrast in a manner similar to that described for conventional TEM. However, the prevailing imaging mode for STEM is high-angle annular dark-field (HAADF). This is based on a ring-shaped detector collecting electrons scattered by angles between several tens to about one hundred milliradians. In this mode, the interference effects between electron states responsible for the complex contrast behavior in conventional TEM do not show up in the images because their contributions are effectively averaged out over the detector area (*30*, *31*). What remains is an intensity modulation resembling that formed by electron waves moving through the specimen as bound states along laterally independent atomic columns. The corresponding absence of lateral interference effects means that the observed contrast arises from incoherent scattering. The great advantage of this imaging mode is that the incoherent intensity distributions can be understood directly in terms of atomic structure. Because the scattered intensity depends on the nuclear charge number *Z*, allowing different atomic species to be distinguished, the HAADF mode is therefore also referred to as *Z*-contrast imaging.

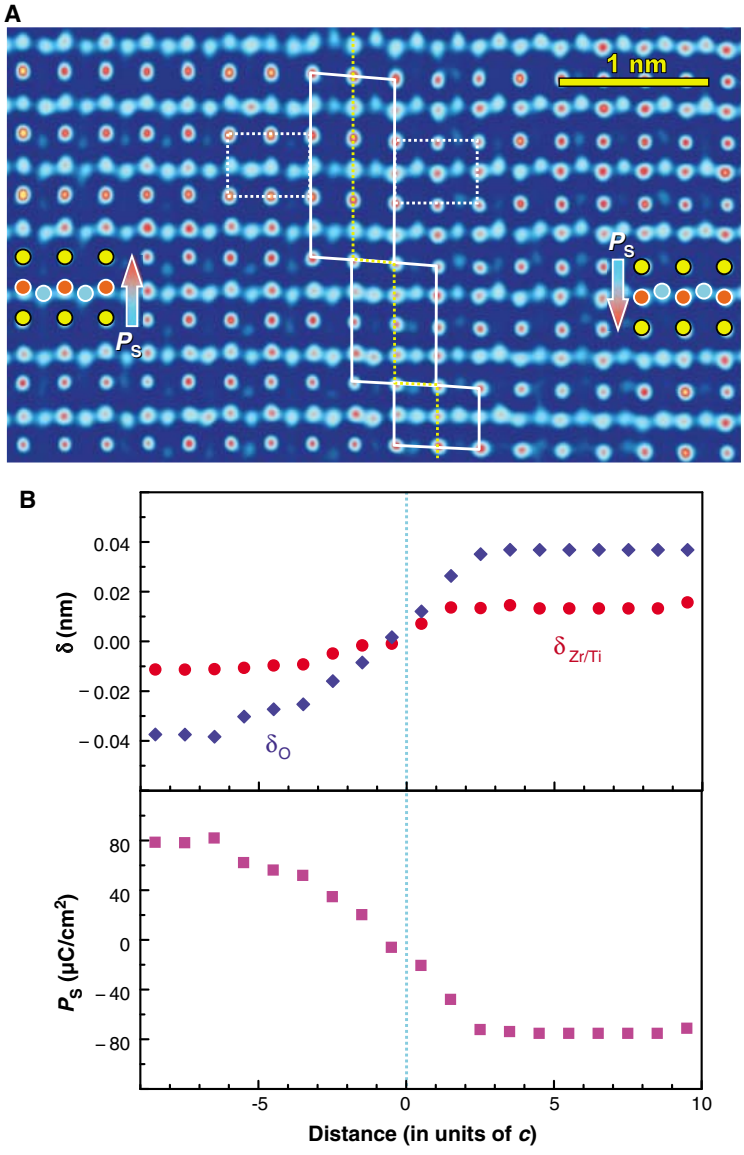

Fig. 3. (**A**) Transversal inversion polarization-domain wall in ferroelectric PZT. Arrows give the direction of the spontaneous polarization, which can be directly inferred from the local atom displacements. The shifts of the oxygen atoms (blue circles) out of the Ti/Zr-atom rows (red circles) can be seen directly, as well as the change of the Ti/Zr-to-Pb (yellow circles) separation. (**B**) Atomic resolution measurements of the shifts of oxygen (δ_O), and titanium/zirconium ($\delta_{Ti/Zr}$) atoms in a longitudinal-inversion domain wall and the value of the local polarization *P* that can be calculated from these data [adapted from (*29*)].

The spatial-intensity profile of the probe is, in a manner equivalent to conventional TEM, related to the point spread induced by the aberrations of the probe-forming optics. Correcting the spherical aberration allows the beam diameter to be reduced; thus, higher resolution is achieved (*32*–*34*). A study on GaN and AlN quantum well structures demonstrated that both resolution and contrast determine the eventual result. In this study, the direction of the electric polarization field resulting from locally uncompensated electronic charges could be derived by quantitatively evaluating the asymmetric Al-N pair contrast (Fig. 4A). In spite of the low probe diameter of about 0.07 nm, the 0.011-nm-wide Al-N pairs could not be resolved as two well-separated maxima because the high-*Z* aluminum dominated the contrast, partly obscuring the low-*Z* nitrogen (*35*). In a study of a silicon crystal along a crystallographic [112] direction, the atom-pair separation of 78 pm was clearly resolved (Fig. 4B) (*36*). The direct interpretability of the HAADF images in terms of atomic structure enabled the discovery of a new type of reconstruction of the NiS_2/Si(001) interface (*37*). In another study of platinum trimers on alumina surfaces, high-resolution measurements in aberration-corrected HAADF allowed single atoms to be imaged

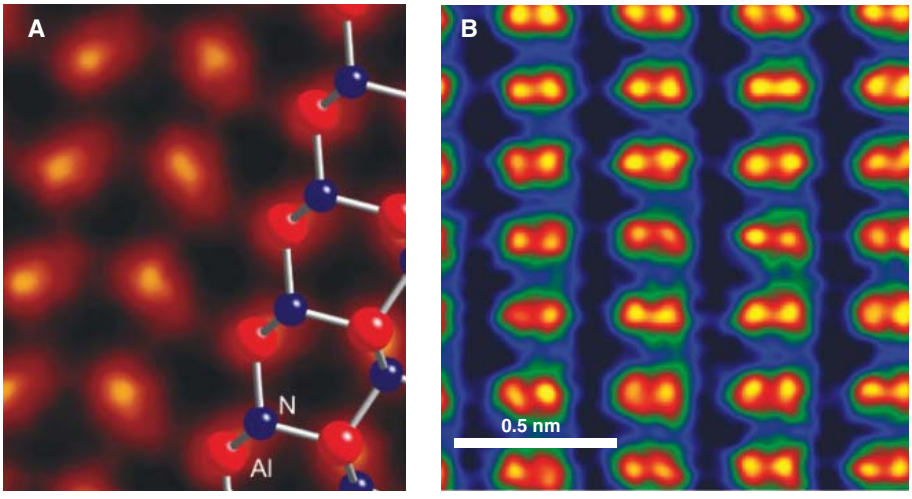

Fig. 4. HAADF images in STEM. (**A**) AlN in [–2,1,1,0] projection. Although the close Al-N pairs, 0.11 nm apart, cannot be resolved as separated entities in the 120-keV instrument, the contrast allows, as confirmed by quantum mechanical calculations, the identification of the bright maximum as Al whereas the tail belongs to N (see ball-and-stick model). This asymmetry can be used to determine the local lattice polarity [from (*35*)]. (**B**) Si along a crystallographic [112] direction. The atom-pair separation of 78 pm is clearly resolved in the 300-keV instrument [adapted from (*36*)].

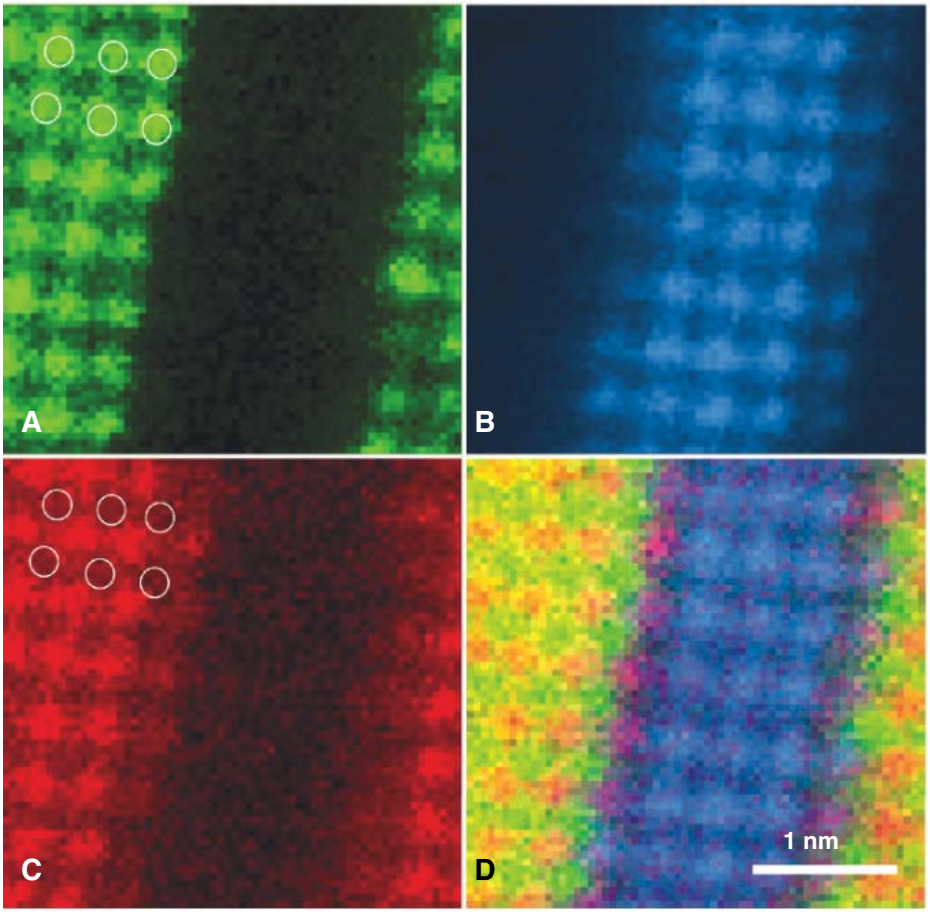

Fig. 5. Spectroscopic imaging of a $La_{0.7}Sr_{0.3}MnO_3/SrTiO_3$ multilayer showing the different chemical sublattices in a 64-×-64-pixel spectrum image extracted from 650-eV-wide EELS data recorded at each pixel. (**A**) La-M edge. (**B**) Ti-L edge. (**C**) Mn-L edge. (**D**) Red-green-blue false-color image obtained by combining the rescaled Mn, La, and Ti images. The purple color at the interface in (D) indicates Mn-Ti intermixing at the Ti sublattice. White circles indicate La atom columns [adapted from (*41*)].

and different chemical compounds to be differentiated (fig. S3) (*38*).

The annular detector used for HAADF allows the low-angle scattered electrons to pass. These electrons can be used for EELS, using a spectrometer placed beneath the detector plane. This configuration permits an atomic-scale–resolution analysis of the electronic structure by line traces or by two-dimensional mapping (*39*, *40*). Spherical-aberration correction enables the aperture angle of the probe to be opened up, thus increasing the beam current by several orders of magnitude, which is particularly advantageous for chemical mapping (*41*). Because the scattering cross sections for inner-shell ionization processes are relatively low, dwell times (for the acquisition of one pixel of data) for obtaining adequate counting statistics are comparable to the time scale on which specimen drift and other instabilities manifest themselves. In a recent-generation aberration-corrected instrument, the total acquisition time was only 30 s for an atomic-resolution 64-×-64-pixel 450–eV-wide high–signal-to-noise EELS spectrum image of $La_{0.7}Sr_{0.3}MnO_3/SrTiO_3$ multilayers (Fig. 5) (*41*). This demonstrated an increase in signal intensity and a corresponding reduction in dwell time by about two orders of magnitude as compared with uncorrected instruments (*42*). The high quality of these data allow chemical bonding states to be explored on an atomic scale.

With probe sizes becoming smaller, the spatial resolution becoming higher, and EEL investigations becoming more quantitative, higher-order effects neglected in earlier treatments have to be considered. First of all, this concerns the depth dependence of the quantum mechanical localization of the probe's electron wave field in the sample potential. Interpretation of spectrum images is further complicated by the nonlocal nature of the core-loss electron-interaction potential (*43*, *44*). As a result, the local intensity distribution in a spectrum image may deviate considerably from the geometry of the atomic sites responsible for a specific energy loss. This makes the interpretation of results of studies of bonding variations and modifications of the electronic density of states at defects and interfaces more difficult. Therefore, theoretical modeling, similar to that in quantitative conventional TEM, is now considered an essential part of quantitative high-resolution EELS mapping.

The Future

Developments in electron optics are continuing to improve the resolution even further, beyond that of the present generation of aberration-corrected instruments. These efforts are, above all, driven by an increasing demand for instrumentation, allowing high-precision measurements to characterize the structural, physical, and chemical properties of synthetic nanometer-scale structures, such as clusters, organic and inorganic fullerenes, carbon nanotubes, epitactic heterostructures with particular layer and interphase properties, and interface engineering, to produce materials with

new synthetic electronic properties. A very active field that is benefiting substantially from modern EM techniques is oxide electronics (*45*, *46*), which has recently been highlighted by the discovery of superconductivity in the interface of the two insulating perovskites $LaAlO_3$ and $SrTiO_3$ (*47*). In all these nanostructures, local atom displacements on the picometer scale and subtle changes in the electron bonding configuration occur at interfaces over distances of atomic dimensions. These generally are outside the range of even the most sophisticated x-ray–scattering techniques used for structure investigations. In addition, subtle changes in electronic structure and bonding occur on the atomic scale. As compared with TEM, synchrotron-radiation experiments offer higher energy resolution, but their spatial resolution is lower by several orders of magnitude.

A resolution of 50 pm was recently claimed in factory tests of an instrument developed within the U.S. Department of Energy's Transmission Electron Aberration-corrected Microscope (TEAM) project. The TEAM instrument offers both TEM and STEM operation and employs an improved spherical-aberration corrector technology together with a monochromator in the electron-beam forming system. In a second stage of development, an additional corrector for chromatic aberrations (*48*) will be implemented. The availability of chromatic in addition to spherical correction will not only further improve the image quality, it will also allow the construction of large-gap pole-piece lenses. These will provide sufficient space around the specimen position for the accommodation of micromanipulation devices, allowing in situ dynamic studies under direct observation. Examples are structural transformations during heating or cooling or chemical reactions in a controlled gas atmosphere. Another field of development concerns the improvement of spectral electron-energy resolution. Although commercial energy-loss spectrometers offer a resolution of typically 100 meV, a resolution of 55 meV has recently been claimed in the German Sub-Electronvolt Sub-Ångström Microscope (SESAM) project. This improved resolution can be exploited to study small changes in the width of the local electron-energy band-gap near interfaces in multilayer semiconductor nanodevices (*49*).

In most cases, atomic-resolution TEM is used to obtain a two-dimensional projection of the specimen structure averaged along the electron propagation direction. Although not entirely excluded, elucidating structural and chemical variations along the depth coordinate turns out to be quite difficult in practice. In fact, the scattering effects induced by single atoms are difficult to trace back to a particular depth position in the sample by using quantum mechanical calculations. Three-dimensional electron tomography attempts to overcome this limitation (*50*). In a recent study on octahedral MoS_2 fullerenes, it was shown that the NCSI technique has the potential for enabling tomography with three-dimensional atomic resolution (*51*). Another technique for obtaining information in three dimensions is based on the fact that the large probe aperture angles in STEM achieved by aberration-corrected optics result in a substantially reduced depth of focus; in principle, this allows depth-sectioning of samples (similar to confocal light microscopy) (*52*). This technique has been successfully used to image single Hf atoms at a depth resolution of about 1 nm in amorphous SiO_2 (*53*).

A recent study in which gold nanoparticles were imaged at atomic resolution by electron holography indicates that this technique benefits from a remarkably increased signal-to-noise ratio when carried out with an aberration-corrected instrument (*54*). On the other hand, electron holography offers the potential to measure, in addition to the structure, local electrical polarization fields on the atomic scale—for example, in nitride-heterostructures or in ferroelectric domain boundaries.

The ultimate quantum mechanical limit to resolution is set by the width of the electron wave function inside the specimen. Because of Heisenberg's uncertainty principle, the extension of the wave function is always larger than the width of the scattering potential, which makes the limit a function of nuclear charge (*55*, *56*). For silicon ($Z = 14$), the width of the wave function of 300-keV electrons is about 70 pm. This is close to what is observed with present-day optics. Therefore, it mainly will be the heavier elements that are expected to benefit from further progress.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/321/5888/506/DC1
Figs. S1 to S3
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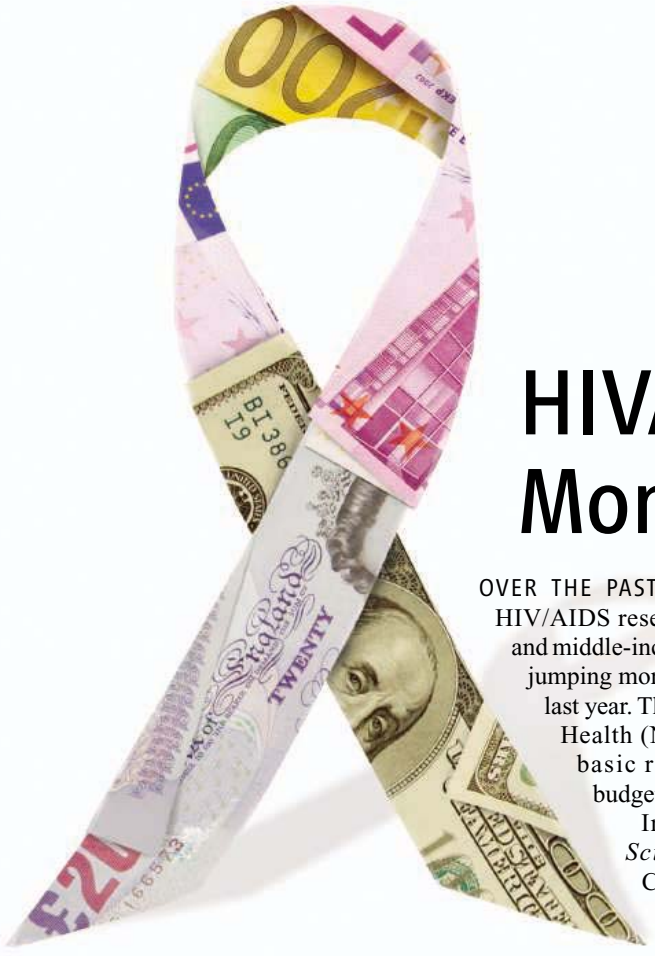

HIV/AIDS: Money Matters

OVER THE PAST DECADE, FUNDING FOR HIV/AIDS research and treatment in low- and middle-income countries has exploded, jumping more than 20-fold to $10 billion last year. The U.S. National Institutes of Health (NIH), the largest funder of basic research, also doubled its budget to nearly $3 billion.

In a 6-month investigation, *Science* correspondent Jon Cohen followed the money, looking at how these dollars have been divvied up and what they have accomplished (see overview, p. 512), how countries have dealt with this sudden influx of cash (with a case study of Botswana, p. 526), and who's minding the store and what happens when abuses occur (as in Uganda, p. 522).

He found that the billions of dollars the major funders have spent have been concentrated in a few countries—often for legitimate reasons—but not necessarily in the countries with the worst epidemics. And despite the massive influx of funds, the number of people in need of anti-HIV drugs continues to climb: a reflection of the treatment's success but also of the failure of prevention efforts. All of this raises unsettling questions about whether resources can keep up with future demands.

A separate analysis looks at who has received the most NIH money and which authors and institutions have published the highest-impact papers (p. 518).

This issue appears on the eve of the XVII International AIDS Conference, which will run from 3 to 8 August 2008 in Mexico City. The conference motto is "Universal Action *Now*" (see the Editorial by Soto-Ramirez, p. 465). Action requires funding. But as this package of stories emphasizes, money alone does not equal effective action.

Strategic rethinking has to be a priority, as shown in two Perspectives. Grant *et al.* (p. 532) discuss the ways in which poor choices and poor coordination have led to wasted efforts and recurrent crises in microbicide research. Fauci *et al.* (p. 530) look at recent disappointments in HIV vaccine research and ways to reinvigorate the field.

–LESLIE ROBERTS AND BARBARA JASNY

A Kaiser Family Foundation (www.kff.org) Media Fellowship helped support Jon Cohen's reporting for this project. Kaiser also maintains a useful Web site about HIV/AIDS funding (www.globalhealthfacts.org). More detailed information about this package of stories, including video documentaries, can be found on *Science* Online (www.sciencemag.org/aids2008).

HIV/AIDS: Follow the Money

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Perspectives

See also Editorial p. 465 and online material on p. 459 or at www.sciencemag.org/aids2008

Science

The Great Funding Surge

HIV/AIDS now attracts billions of dollars each year, extending millions of lives. But many remain untreated and, without better prevention, the money will soon fall short

A DECADE AGO, WHEN THE WEALTHY COUNTRIES OF THE WORLD reached into their pockets to help the less fortunate combat HIV/AIDS, they came up with a mere $485 million. Last year, the HIV war chest for these developing countries, including some money from their own budgets, totaled $10 billion, more than a 20-fold increase.

A variety of factors contributed to this extraordinary surge in funding, but one stands out: success with anti-HIV drugs. Drugs made little headway against the virus in the decade following the proof in 1984 that HIV causes AIDS. But in 1994, a study showed that AZT could prevent transmission from an HIV-infected pregnant woman to her baby. Then in late 1995, researchers reported that potent anti-HIV drugs used in combination could stave off AIDS and improve the health of those already sick. By the late 1990s, studies in Thailand and Uganda had shown cheaper, simpler ways to prevent mother-to-child transmission (MTCT) with anti-HIV drugs, and Brazil and Haiti demonstrated that if the will existed, treatment could reach rich and poor alike. But for tens of millions of people, that wasn't happening.

At the 2000 international AIDS conference in Durban, South Africa, the issue of "universal access" came to a full boil, and the next year, the United Nations General Assembly held a special session on HIV/AIDS, sparking a global effort to increase access to, and reduce the costs of, anti-HIV drugs. Billions of new dollars from new sources began flowing into poor countries. First came the World Bank's Multi-Country HIV/AIDS Program (MAP) in 2000 that spread grants and loans with generous terms to many hard-hit spots. In 2002, the Global Fund to Fight AIDS, Tuberculosis, and Malaria—a partnership of governments, philanthropies, civil society, industry, and affected communities—opened its doors to all comers and, in a novel twist, asked governments to detail their needs and linked payments to performance. U.S. President George W. Bush the next year launched the President's Emergency Plan for AIDS Relief (PEPFAR),

Online
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Related videos and podcast interview with the author.

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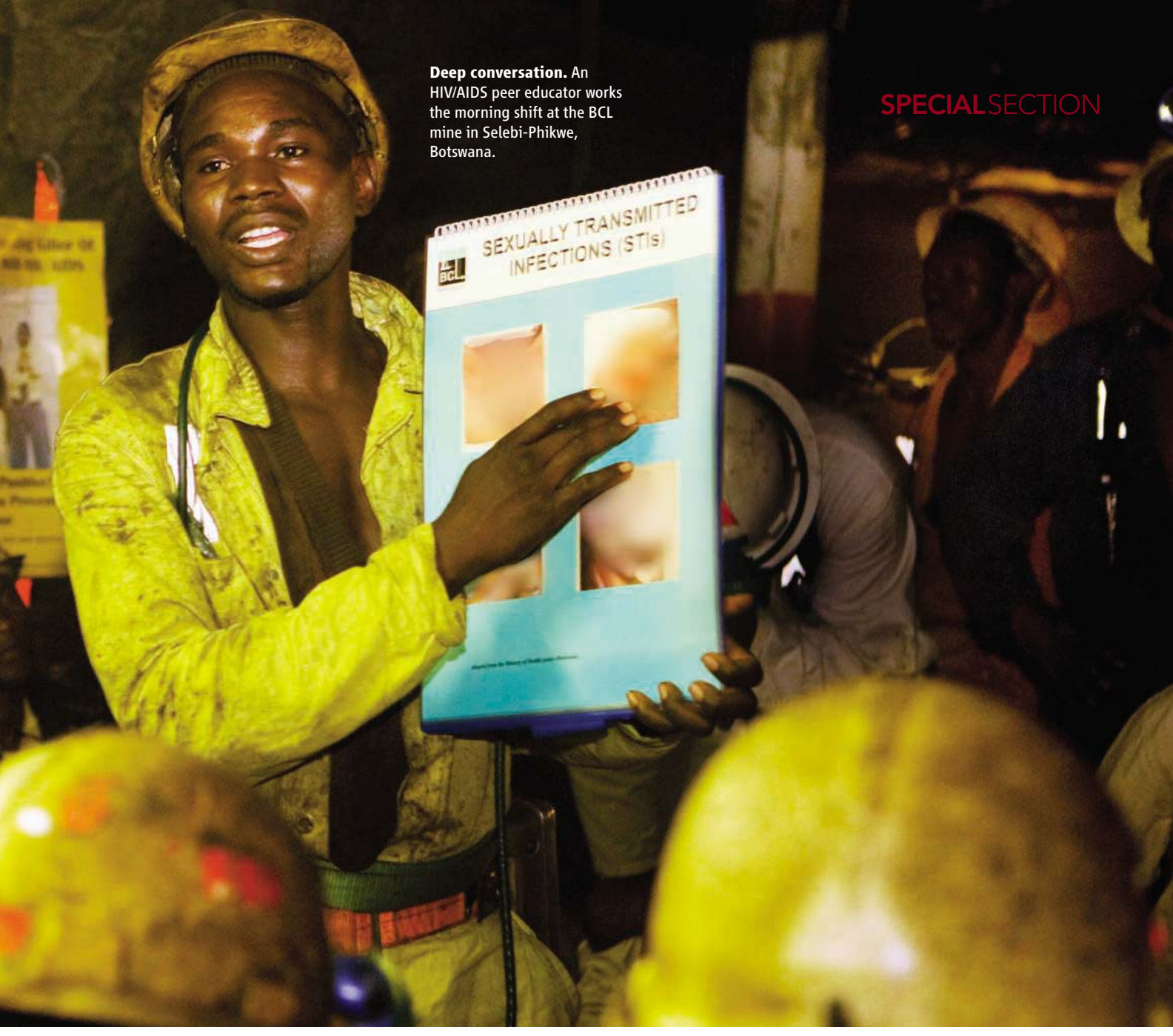


Deep conversation. An HIV/AIDS peer educator works the morning shift at the BCL mine in Selebi-Phikwe, Botswana.

which quickly ramped up treatment and prevention efforts in 15 "focus" countries. The Bill and Melinda Gates Foundation made HIV/AIDS a priority, investing nearly $2 billion in treatment, prevention, and research to help the poor. In 2006, the General Assembly upped the ante and declared that every HIV-infected person in need of treatment should receive it by 2010. The response "has transformed … how the developed world partners with the developing world in addressing a catastrophic health crisis," says Anthony Fauci, an architect of PEPFAR and head of the U.S. National Institute of Allergy and Infectious Diseases (NIAID).

HIV-infected people around the globe are now living longer, and several communities have even seen their epidemics start to wane. "Seven years ago, there wasn't a single African on treatment from a donor program in a global pandemic of unprecedented proportion," says Jeffrey Sachs, a health economist at Columbia University who in 2000 called the underfunding of AIDS "perhaps the most shocking feature of our generation." Today, Sachs has toned down his chiding of the wealthy world and even applauds some of its actions. "Now there's finally a process in place and a glimmering that the epidemic is peaking. Incidence is coming down in some places, treatment is steadily increasing, and health care systems are beginning to rise from nothing."

But serious problems remain. Many countries, short on health care workers and infrastructure, have had difficulty building prevention and treatment programs. Several "donor darlings" have received the lion's share of aid money, while some politically troubled countries have received scant help. The Global Fund has suspended or canceled grants because of corruption. Current funding levels won't come close to providing universal access by 2010, and high rates of new infections—an indication that prevention programs are having limited success—are placing an ever-increasing demand on treatment programs. The vaccine search, the best hope for slowing HIV's spread, has failed so miserably that leaders in the field recently persuaded Fauci to shift millions slated for clinical trials to more fundamental studies. Several microbicides (topical gels to prevent transmission) similarly have proven

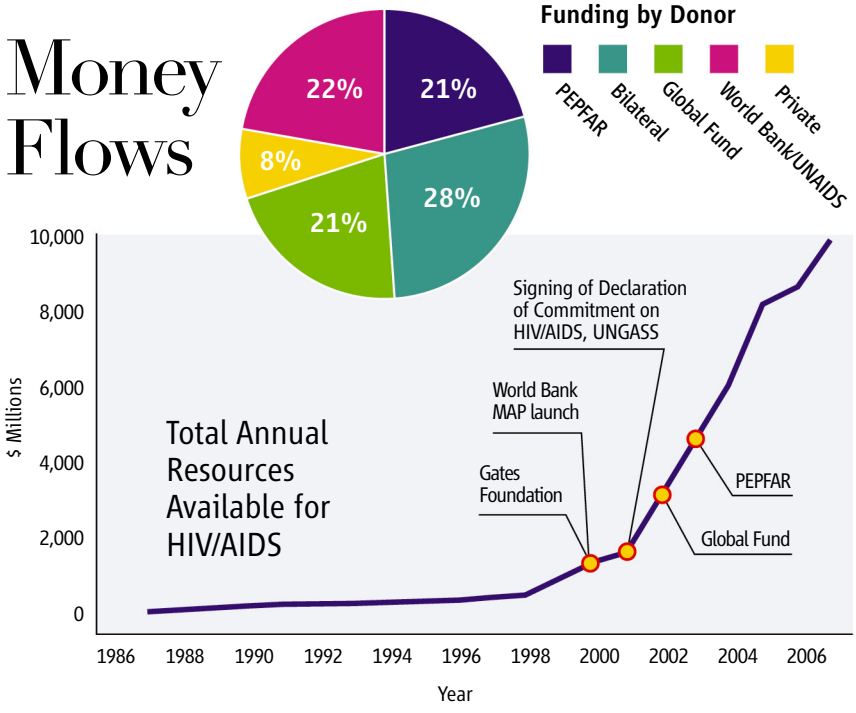
UPPING THE ANTE

M = million
n/a = not available
bold = PEPFAR-funded countries

Disparities

		ADULT PREVALENCE (%)	POPULATION HIV+ (M)	TOTAL POPULATION (M)	DISBURSED ($M)	COMMITTED ($M)	DOMESTIC ($M)	DISBURSED/POP HIV+ $/Person
1.	Myanmar	1.3	0.36	47.40	14.4	14.4	n/a	40.00
2.	CAR	10.7	0.25	4.30	21.6	57.4	0.6	86.40
3.	Zimbabwe	20.1	1.70	12.31	167.2	195.8	63.4	98.35
4.	DRC	3.2	1.00	65.75	134.8	284.9	3.6	134.76
5.	**South Africa**	18.8	5.50	44.00	936.4	1007.2	480.2	170.25
6.	Angola	3.7	0.32	12.30	57.0	143.5	39.0	178.13
7.	**Kenya**	6.1	1.30	36.91	995.4	1143.8	33.2	765.68
8.	**Uganda**	6.7	1.00	30.26	837.5	871.7	12.3	837.52
9.	**Botswana**	24.1	0.27	1.80	266.3	275.9	130.8	986.30
10.	**Ethiopia**	2.1	0.98	76.51	848.9	1160.4	n/a	868.53
11.	**Namibia**	19.6	0.23	2.10	267.5	320.0	38.5	1162.96
12.	**Rwanda**	3.1	0.19	9.90	383.0	423.2	4.3	2015.79

Money Flows



worthless—or even harmful—in large, expensive human studies. In addition, serious questions have emerged about whether all this new money for HIV/AIDS has come at the expense of meeting other pressing health needs.

During the past 6 months, *Science* has examined how the dollars are being distributed and spent. Which countries, institutions, and researchers have received the largest slices of the pie (see p. 520)? Who has been left out? Which efforts have gotten the most bang for the buck (see p. 518)? What happens when corruption surfaces (see p. 522)? What lessons can governments, clinicians, and communities learn from the smart investments, missed opportunities, and outright mistakes made by other countries (see p. 526)? And how sustainable is the massive treatment rollout now under way?

Hard answers remain elusive, but a few common themes have emerged. Money alone does not solve every problem, but it sure helps. Unfortunately, no master logic matches the distribution of aid to those most in need. Money can do good only if it's spent in the right ways, and many agree that prevention, in particular, has been shortchanged. For each person who starts treatment, nearly three people become infected, according to recent estimates. "This is not something where you can say, 'Okay, I fixed it,' and walk away," cautions Fauci. "You have to make a long-term commitment with a lot of resources, and it will only be successful if you can bring down the infection rate to a dramatically lower level than it is now. It's a phenomenal challenge."

Fair share?

The Global Fund, PEPFAR, the World Bank, and the Gates Foundation have contributed the bulk of the funds to help countries cope with HIV/AIDS. An analysis by *Science* combined all the money contributed since 1989 by these four sources to the 29 countries with the most HIV-infected people. (The analysis omits China, India, Russia, and Indonesia because they have large populations and relatively low HIV prevalences, which means that their prevention and treatment needs differ markedly from those of the other countries.) In committed funds, the 15 PEPFAR focus countries—which account for about half of the HIV-infected people in the world—have received more than 80%. Ethiopia, a PEPFAR country that reports a relatively low HIV prevalence of 2.1%, has more committed money than any other country. Looking at disbursed dollars per HIV-infected person puts the disparities in

SOURCES: UNAIDS, PEPFAR, GLOBAL FUND, GATES FOUNDATION, WORLD BANK, UNGASS

a harsher light still. Rwanda, a PEPFAR country, has received $2015 per infected person. Disbursed funds to the Democratic Republic of the Congo (DRC), the Central African Republic, Myanmar (formerly Burma), Sudan, and Zimbabwe amounted to less than $150 per infected person; these five countries together have 3.7 million infected people—more than 10% of the global total.

The universal-access ethos contends that everyone, everywhere should benefit equally from anti-HIV drugs, and the funding surge has ended the era in which the rich had access and the poor did not. But the billions of new dollars have created a new division of haves and have-nots within the developing world. "This large imbalance exists," says epidemiologist Robert "Robin" Ryder of the University of California, San Diego, who co-runs the Central African portion of the U.S. National Institutes of Health–funded International Epidemiologic Database to Evaluate AIDS. As Ryder points out, six of the top 10 recipients of disbursed Global Fund money are also PEPFAR focus countries. "The rich get richer and the poor get poorer," he says.

Donors have sound reasons for offering little aid to some countries that have large HIV/AIDS epidemics: Corrupt governments, civil wars, and the absence of infrastructure increase the likelihood that money will be wasted. And many of the most fragile states have shaky relations with the biggest donors, including the United States, Europe, Japan, and Australia. "Foreign aid is an extension of foreign policy," says Peter Piot, head of the Joint United Nations Programme on HIV/AIDS (UNAIDS). "It's an illusion that it's based on objective criteria. That's why I'm a big supporter of the Global Fund. It's far less influenced than others."

But unstable countries also have a distinct disadvantage with the Global Fund, Piot concedes, as they are less likely to be able to submit strong proposals. "We're actually funding to the maximal level that the country is asking and the level in which it can bring evidence that it can make the money work," says Michel Kazatchkine, head of the Global Fund. "And of course the weaker the country, the greater the gap will be between demand and need." Kazatchkine adds that the best funded countries continue to struggle, too: "Even in the countries that are darlings of Global Fund and PEPFAR and Gates, … these amounts of money are below the actual need."

Stefano Bertozzi, a health economist at the National Institute of Public Health in Mexico City, says the distribution of money is more rational today than in the past, but he argues that the "funding orphans" deserve serious attention. "We need to make sure countries in greatest need have access to the funds," he says. To this end, Aidspan, a Global Fund watchdog based in Nairobi, proposed in an April report that a mentoring program of independent consultants be set up to help countries conceptualize but not write their proposals. "The Global Fund has no mechanism for saying publicly or privately, 'The gap is huge in country X and small in country Y: What can we do to get more money to country X?' " explains Bernard Rivers, who heads Aidspan (see sidebar, p. 524). "They're very hands-off. They can't say, 'Help us to help you to write a great proposal so we can get you a lot of money.' "

Measuring impact

Donors and researchers have tried to assess the impact of the funding surge and disentangle the overlapping investments. A few clear success stories have emerged, such as prevention of MTCT and reduction of deaths in Botswana. But hard data about reduction in new infections from sexual transmission, and direct evidence that mortality and morbidity have dropped on a country level because of anti-HIV drugs, are the exception to the rule.

PEPFAR and the Global Fund regularly report results of their investments, and the World Bank does periodic assessments. The Gates Foundation, a private philanthropy that does not have to answer to constituents, offers little information about the impact of its programs.

As of June 2008, PEPFAR says it has prevented 7 million infections,

Clean shot. This heroin user in Chennai, India, had access to clean needles, but many don't.

CREDIT: MALCOLM LINTON

trained some half-million care providers, and "supported" the antiretroviral (ARV) treatment of 2 million HIV-infected people and the care of 10 million others. The word "support" underscores that PEPFAR often cannot separate its contributions from those made by the Global Fund and domestic spending.

The same month, the Global Fund reported that it had provided anti-HIV drugs to 1.75 million people, helped 2.8 million orphans, provided counseling and testing to 46 million people, and trained 7.6 million people to provide care for HIV/AIDS, TB, and malaria in 136 countries. The World Bank has calculated that 40% of its MAP funds in Africa have gone to "strengthening systems," which includes everything from training staff of nongovernmental organizations (NGOs) to building infrastructure to improving the supply chain of drugs and testing kits. About one-third of the bank's money went to prevention efforts, "contributing" to the enrollment of some 1.5 million HIV-infected pregnant women in programs to thwart transmission to their babies. Only 16% of bank funds supported care and treatment, accounting for a modest 27,000 people on anti-HIV drugs by the end of 2005.

Last year's annual UNAIDS update illustrates the difficulty of determining on a country level the impact of funding. Kenya, which has received ample support from PEPFAR and the Global Fund, had sharp drops in prevalence that appeared in part related to changes in behavior. But so did Zimbabwe, which is not a PEPFAR focus country, has received relatively little from the Global Fund, and, all told, has taken in one-seventh as much aid per infected person as Kenya. Roeland Monasch, a UNICEF representative in Zimbabwe who helped that country prepare a successful Global Fund proposal, says one of the factors accounting for the drop may be the economic crisis. "There's much less mobility of people in the country, and men can't afford girlfriends and to travel up to the country in the weekend," says Monasch.

Ryder contends that the skewed funding to the darlings also undermines attempts to assess the effectiveness of ARV treatment in lowering mortality rates. He notes that PEPFAR focus countries such as Rwanda have the resources to track people who are on anti-HIV treatment, whereas poorer countries such as the DRC don't. "It really affects assumptions about efficacy," says Ryder. Say researchers in Rwanda discover that a woman on treatment died from AIDS, whereas if that same woman lived in the DRC, she might be "lost to follow-up" and excluded in the final analysis. "It's paradoxical," says Ryder. "The more money you put into a country, the more likely you are to show a higher death rate because you have the wherewithal to know someone died."

Strengths and weaknesses

The "big three" publicly funded HIV/AIDS efforts—the Global Fund, PEPFAR, and the World Bank's MAP—have all received intense scrutiny—but PEPFAR has attracted by far the most, in part, says Bertozzi, because of initial skepticism about an ambitious new program created by Bush, who many thought had shown little interest in the HIV/AIDS epidemic.

PEPFAR has won over many doubters because of the speed with which it delivers treatment and prevention. Yet some of PEPFAR's policies have dismayed researchers and HIV/AIDS advocates, who contend that they promote Bush's religious and political agendas while disregarding scientific evidence on which interventions work best. In particular, critics have assailed PEPFAR for emphasizing abstinence-only education, which they say does little good, and requiring recipients to sign a pledge against prostitution that they say

Recognizing reality. Peru acknowledges that men who have sex with men are at high risk—and recruits them for an AIDS vaccine study.

CREDIT: MALCOLM LINTON

further stigmatizes sex workers and discourages prevention outreach efforts geared toward them.

Another concern is that PEPFAR funnels much of its aid through international NGOs—big winners are the U.S.-based Family Health International and Management Sciences for Health—instead of trying to strengthen in-country capacity. In an April 2008 report, epidemiologist Nandini Oomman and colleagues at the Center for Global Development, an independent think tank in Washington, D.C., noted that about 70% of PEPFAR funds have gone to international organizations, many of them faith-based. Says Oomman, "If you're thinking about a sustainable long-term response, you really do have to think about how you develop local capacity."

PEPFAR's head, Mark Dybul, concedes that some investments are skewed to international NGOs, but he says that's what an effective emergency response requires. "You don't respond to a tsunami by just sending money to a country and hoping that they take care of all the problems," says Dybul. And he says that in countries like Uganda that have "more mature" epidemics, this skewing doesn't occur because they have had time to develop their local response. "It takes a while to build capacity," Dybul says.

As *Science* went to press, Congress was expected to reauthorize PEPFAR for the next 5 years, tripling its budget and doing away with some of the most restrictive requirements. The draft law also calls for training and retaining 140,000 new health care workers. Dybul says PEPFAR may decide to make substantial investments beyond the original 15 focus countries.

In contrast to PEPFAR, the Global Fund is more hands-off, seeing itself as little more than a financing mechanism. "Our funding is based on a fundamental principle: country ownership," says director Kazatchkine. Yet some contend that the fund would have more impact if it had people in countries who helped, as PEPFAR and the World Bank do.

Country ownership sometimes trades autonomy for economy. Consider the purchase of drugs, which Global Fund recipients negotiate independently. Epidemiologist Brenda Waning of Boston University School of Public Health says there is a "ridiculous" variability in how much different Global Fund recipients pay for anti-HIV drugs. "Some countries are paying 15 times as much as other countries," says Waning. "The Global Fund, from what I can tell, doesn't seem as though they bother looking at prices people are paying."

The World Bank's MAP program, too, has struggled to conduct proper evaluation and monitoring and has created overly cumbersome procedures to disburse money, according to two in-depth analyses published by the bank in 2005. These sharply self-critical reports described the impact of the bank-sponsored projects as "uneven" and also faulted MAP for "grossly" neglecting research to evaluate the cost-effectiveness of different interventions.

The big three are all still fairly young, and the introspection and outside criticism continues to push each one to improve. But health economist Bertozzi says more attention should be paid to how they fit together. "It's a shame that there's no demand to look at the relative strengths and weaknesses and efficiencies of funding HIV programs through three different models," says Bertozzi. "Probably the most efficient way to spend the money is some mix of those three."

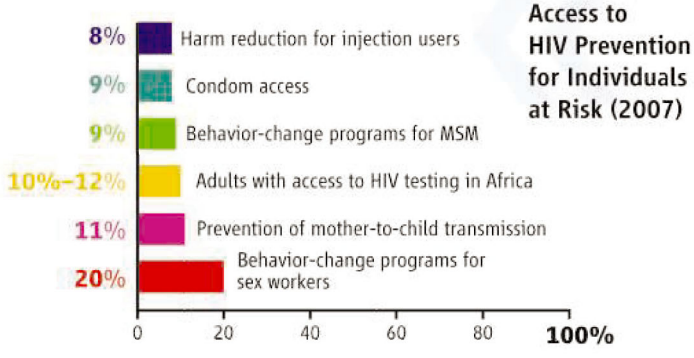
Never-ending

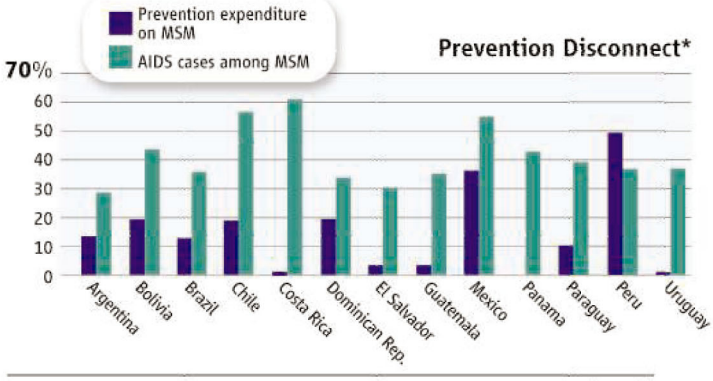
Even if they differ on which funding mechanism works best, experts

Hands-on training. A woman at a brothel in Guatemala City shows an outreach worker that she's condom savvy.

Prevention

Prevention Expenditures and Needs in 38 Countries, 2006
($ MILLIONS)





* MSM drive epidemics in many Latin American countries, but in 2001 few made substantial investments in prevention for that high-risk group.

(PHOTO) MALCOLM LINTON; (SOURCES): UNAIDS, SIDALAC, UNICEF, WHO

Bang for the Buck

Three modest NIH investments yield big dividends

Much of the nearly $3 billion the U.S. National Institutes of Health (NIH) spends on HIV/AIDS research each year supports basic studies that may not yield insights for decades. But three relatively modest investments continue to advance the field in ways large and small, year after year. Together, they consume about $20 million a year—not even 1% of the entire budget—and help thousands of researchers around the world at no cost. "I think of these three as the gifts that keep on giving," says Anthony Fauci, head of NIH's National Institute of Allergy and Infectious Diseases (NIAID) in Bethesda, Maryland, which funds these projects.

NIH AIDS Research and Reference Reagent Program

Annual budget: $3.75 million
Location: Germantown, Maryland

Say you're running an experiment that calls for some HIV in the flavor known as subtype C. Or maybe it's a dash of the protease inhibitor indinavir, a dollop of a monoclonal version of the b12 antibody, or a sprinkle of the human version of the APOBEC3B protein. Each of these reagents is yours, free, aside from a nominal shipping fee.

Since 1988, the NIH AIDS Research and Reference Reagent Program has asked investigators to share interesting samples of HIVs, antibodies, peptides, antivirals, cell lines, proteins, and expression vectors. A nondescript office building in an aging business park now warehouses 8500 unique reagents. Last year, the program sent out 15,800 small samples of reagents to nearly 4000 scientists in 65 countries, often within a week.

Opendra Sharma of NIAID, who runs the program, says researchers typically are "very happy" to supply samples as long as recipients agree not to use them for commercial purposes. And when Sharma and his team run across someone who does not want to share, they have their methods of persuasion, he says: "We twist their arms, but we don't break them."

A recent survey of nearly 800 users showed 98% or higher satisfaction. But 10% complained that they did not receive *enough* reagents in high enough quantities. As a rule of thumb, investigators are allowed two samples per grant per year, though Sharma says they make exceptions.

Multicenter AIDS Cohort Study

Annual budget: $14.4 million
Location: Chicago, Los Angeles, Baltimore, Pittsburgh

How long does it take to progress from HIV infection to symptomatic disease? What percentage of people live with HIV for more than a dozen years and suffer no harm? Why do some people remain invulnerable to HIV, despite repeated exposure? These are a few of the questions that researchers have probed, thanks to blood samples collected during nearly 25 years by the Multicenter AIDS Cohort Study (MACS). This unusual study has followed thousands of people at high risk of becoming infected with HIV or developing AIDS.

When MACS began in 1984, researchers had yet to prove that HIV causes AIDS. The study at first recruited 5622 gay and bisexual men in four U.S. cities, and each agreed to provide a blood sample and personal history every 6 months. When an HIV antibody test became available the next year, the researchers realized that half the men were infected at the study's start. "The real

agree on one thing: The massive influx of dollars is not keeping up with the pace of the HIV/AIDS epidemic.

In an article in the February 2008 issue of *AIDS*, researchers reported that Uganda, a PEPFAR country and one of the top aid recipients, expects to more than double the number of people receiving anti-HIV drugs between 2005 and 2010. Given the country's projected population growth, however, the number of people who need treatment but aren't receiving it will hardly decline. "It's hard to imagine, at least with the way we're currently doing things, that we can achieve universal access by 2010," says co-author John Stover, a public health analyst who heads the Futures Institute in Glastonbury, Connecticut. "Not only is the number newly in need increasing each year, you need to continue the people on treatment who are surviving." The study bluntly concludes: "Although current prevention and treatment programmes have a measurable effect on the burden of disease, they may not substantially alter the face of the epidemic in the near future."

Using global projections from Stover and others, UNAIDS projects that if prevention and treatment scale-up continues at the same pace as today, by 2010, it will cost $42.2 billion—more than four times the amount available today—to reach 80% (13.7 million people) of those who will then be in need. As of the end of 2007, UNAIDS said 3 million were receiving treatment in low- and middle-income countries, which is about 30% of those who need it.

The only way out of this dilemma, assert Stover, UNAIDS's Piot, and many others, is to invest more in proven prevention interventions and tailor the response to each country's epidemic. "The critical question is not just how much money is available [for prevention], but how much is being spent in the right ways," says epidemiologist Elizabeth Pisani, author of the *Wisdom of Whores*, published earlier this year.

One problem, says Pisani, who previously worked for Family Health International in Indonesia, is the misconception that HIV/AIDS is one epidemic, when in fact it is two: One is in sub-Saharan Africa, where several countries have "generalized" prevalences above 2%; the other is the "concentrated" epidemic in the rest of the world that occurs mainly in high-risk groups such as men who have sex with men (MSM), injecting drug users, and sex workers. For generalized epidemics, everyone above the age of 13 is at risk, she says, and prevention efforts should have a broad agenda. But for concentrated epidemics, it makes more sense to focus on the high-risk groups.

Far too often, says José Antonio Izazola-Licea, who heads the financing and economic division of UNAIDS, countries with concentrated epidemics don't focus their efforts on their high-risk groups. Similarly, says Stover, many countries disproportionately spend prevention money on MTCT even when they have low infection rates and low birth rates. Ten leading AIDS researchers recently argued that not enough effort has gone into promoting male circumcision and the importance of reducing multiple partners (*Science*, 9 May, p. 749).

Prevention efforts directed at high-risk groups can have a major impact in generalized epidemics, too, says epidemiologist Prabhat Jha of the University of Toronto in Canada. In a meta-analysis of 68 epidemiologic studies from Africa in the October 2007 issue of *PLoS One*, Jha and colleagues found that "superspreaders" accounted for a disproportionate

strength of the MACS is we had these men who had the same lifestyle but were uninfected," says John Phair of Northwestern University in Chicago, Illinois, who chairs the MACS executive committee. Basically, the researchers had a built-in control group that enabled them to tease out just how HIV causes AIDS.

Today, 150 MACS investigators follow about 3000 men: Roughly 1600 are infected, and nearly 90% of those are taking anti-HIV drugs. Over the course of the study, investigators have stored more than 1 million samples of plasma, serum, and cells. Culled data, made publicly available, include more than 100,000 entries of CD4 counts—the white blood cells that HIV progressively destroys—and tens of thousands of measurements of the amount of virus in individuals at different times. "The participants give a lot of blood," says Phair. "They're very generous."

The HIV Databases

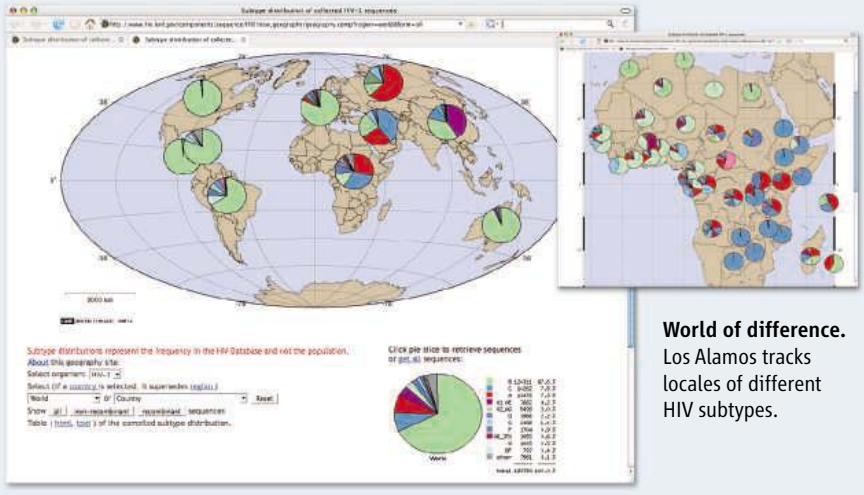
Annual budget: $1.95 million

Location: Los Alamos National Laboratory, New Mexico

Working on GenBank, the genetic sequence database at Los Alamos, Gerald Myers in 1986 became fascinated by the genetic differences between HIV variants. He soon emerged as a world expert on HIV genetic diversity, building a public database that has helped researchers trace the origin of the epidemic, design vaccines, understand viral evolution, track drug-resistance mutations, and even investigate possible skullduggery between competing AIDS researchers.

Myers handed off the project more than a decade ago to immunologist Bette Korber, who added an immunology database that offers some of the most detailed, comprehensive information available about how the virus interacts with immune cells. The immunology database can also interact with what are now almost 250,000 HIV sequences in the older database. Yet another database lets researchers peruse results of all known AIDS vaccine studies done in nonhuman primates. "It's not the quantity of the data but the quality that make the databases stand out," says Korber.

And that quality keeps the quantity of Web site hits high: Last year, the total came to 1.5 million unique visits. **–J.C.**



World of difference. Los Alamos tracks locales of different HIV subtypes.

CREDIT: LOS ALAMOS NATIONAL LABORATORY

amount of transmission even in high-prevalence countries. "The role of superspreaders is still very central to the African epidemic," says Jha.

Epidemiologist Sharon Weir of the University of North Carolina, Chapel Hill, suggests that one way to find superspreaders is to identify where sexual transactions occur—such as truck stops, bars, and street corners—and provide prevention interventions there. People in high-risk groups, notes Weir, often don't identify themselves as MSM or sex workers.

Techniques to identify the newly infected could also slow HIV's spread. In some studies, nearly half of the people who became infected had partners who had recently become infected themselves. These highly infectious people in the acute stage of the disease often have yet to develop antibodies against HIV, but tests exist that can pluck out viral genetic material from blood. In 2005, researchers described how they used a relatively inexpensive technique to identify acutely infected people who gave blood at HIV testing sites (*Science*, 12 August 2005, p. 1002). Basically, they cut costs by removing a small amount of blood from individual vials and pooling them. If a pool tests positive for HIV RNA, they could backtrack and find the acutely infected people, who were then contacted and counseled. Principal investigator Christopher Pilcher of the University of California, San Francisco, is now testing the approach in Brazil, and other groups are trying it in Malawi, Tanzania, and South Africa. Detecting acute infection will be a major prevention strategy, Pilcher says, but cautions that their test is still technically difficult in most developing countries: "The truth is very, very few people are working on this because it has not been identified as a priority."

Displacement?

Amid all the back and forth about how best to provide treatment and prevent HIV transmission, some analysts are arguing that maybe the disease receives proportionally more money than it merits.

In a paper in the 21 December issue of *Health Policy and Planning*, Jeremy Shiffman, a political scientist at Syracuse University in New York, compared donor funding for HIV/AIDS from 1992 to 2005 with money spent on infectious disease, health sector development, and population control. Shiffman found declines in the latter two, leading him to ask whether HIV/AIDS had "displaced" these important public health issues. Shiffman emphasizes that he believes HIV/AIDS doesn't receive *enough* funding. But he thinks global health equity matters, too. "Do we really want a situation where if you have AIDS you get treated and if you're a baby with pneumonia you die? Wouldn't we rather have a health system for both folks?"

Stover of the Futures Institute questions the displacement idea, arguing that money for HIV/AIDS may well be strengthening health systems across the board. He also doesn't rule out the possibility that the wealthy world may devote even more to HIV/AIDS. As Stover sees it, "I'd hate to say, 'No way.' If you asked me 5 years ago whether we'd get to 3 million on treatment, I would have scoffed. One of the things Jeff Sachs has taught us all is because we haven't been able to get that amount of money in the past, it doesn't mean we won't be able to get it in the future."

–JON COHEN

Where Have All The Dollars Gone?

DURING THE PAST DECADE, THE U.S. NATIONAL Institutes of Health (NIH) nearly doubled its annual HIV/AIDS research budget to $2.9 billion (see graph). More than half of that money—$1.6 billion—supports research grants. Of the remaining $1.3 billion, the big-ticket items include contractors ($443 million), intramural NIH research ($288 million), and research centers at academic institutions ($252 million).

To gain a better understanding of the money flow in fiscal year 2007, *Science* asked the NIH Office of AIDS Research which institutions, networks, and individuals received the meatiest portions. These tables frequently overlap: The best funded principal investigators all have links to large collaborations (see "Top Dogs"). Several funding streams also exist, and to simplify this analysis, one table looks at Top Dogs defined by a different metric: researchers who received the most "investigator-initiated" support through the R01 mechanism.

The R01 Top Dogs had 28 grants among them and received $1.95 million to $2.6 million each—four to five times as much as the average AIDS researcher. But many of these R01 Top Dogs ran expensive clinical or epidemiological studies outside of established networks funded by NIH. These networks account for $300 million annually (see "Pack Mentality"). And the 10 best funded universities (see "Fat Cats") run many of them, which helps explain why they rake in about one-fourth of NIH's entire HIV/AIDS research budget.

An analysis of publications, done with the help of Thomson Reuters's Institute for Scientific Information, assesses the impact of the 123,752 HIV/AIDS-related papers published between 1998 and 2007 (see "Territorial Markers"). The average paper was cited nearly 12 times. The single most cited paper shows that potent anti-HIV cocktails led to a steep decline in disease and death in the United States (see "Blue Ribbon"). Researchers who had the most citations per paper, with a minimum of 100 publications, are defined as having had the most impact. And with institutions, money *does* correlate with impact—seven Fat Cats rank in the top 20 in number of cites per 500 papers—but only to a point: Northwestern University has the second-highest citation impact and does not even rank in the top 20 for NIH funding. Money speaks volumes, but it often doesn't have the final word.

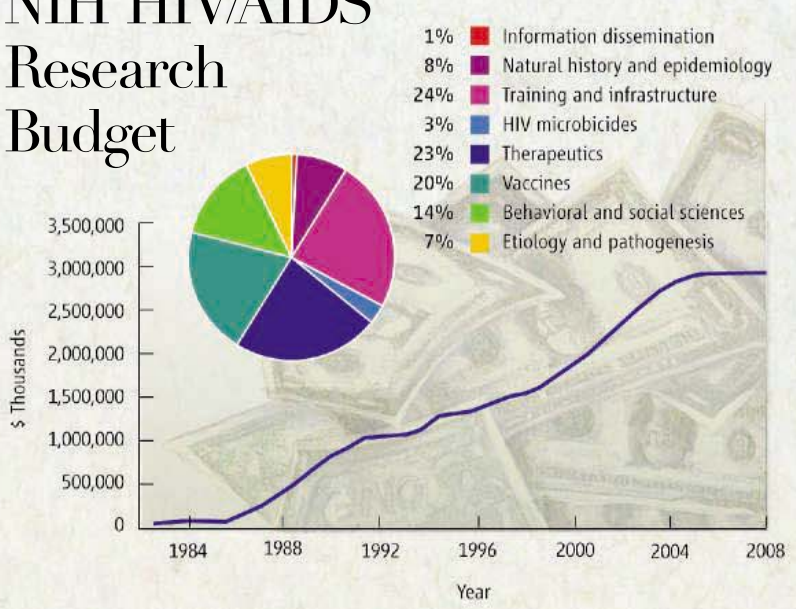
–JON COHEN

Fat Cats

Best Funded Institutions (FY '07)

	$M
U Wash/Hutchinson	138.1
Harvard	103.0
Johns Hopkins	98.3
UC San Francisco	70.8
Duke	68.6
UC Los Angeles	62.6
U Pittsburgh	55.2
U Minnesota	51.5
U Penn	39.0
UC San Diego	32.6
Total	**719.7**

NIH HIV/AIDS Research Budget



SOURCES: NIH/THOMSON REUTERS ISI; ILLUSTRATIONS: BRIAN AJHAR

Top Dogs

NIH-Funded Researchers Who Received the Most in Investigator-Initiated Grants (FY '07)

INVESTIGATOR	INSTITUTION	FOCUS	$M	PAPERS	CITES/ PAPER
Mary Rotheram-Borus	UC Los Angeles	Substance abuse, prevention trials	2.62	117	8.6
David Bangsberg	UC San Francisco	International ARV adherence	2.53	82	20.2
Wendee Wechsberg	Res. Triangle Inst.	Women, substance abuse	2.30	24	8.8
Gregory Kirk	Johns Hopkins	Natural history of IDUs and HIV	2.29	18	4.3
Marc Lallemant	Harvard	Mother-to-child transmission trials	2.28	23	18.4
David Watkins	U Wisc, Madison	AIDS vaccines, monkeys	2.26	80	42.4
Bradford Navia	Tufts	Cerebral injury, clinical studies	2.25	33	11.5
John Jemmott	U Penn	Prevention interventions	2.18	19	22.9
Eric Hunter	Emory	Basic research	2.11	50	35.5
Kenneth Silverman	Johns Hopkins	IDU treatment trials	1.95	12	19.3

Papers 1998–2007; UC = University of California

NIH-Funded HIV/AIDS Investigators With Largest Budgets (FY '07)

INVESTIGATOR	INSTITUTION	NETWORK	$M
Barton Haynes	Duke	CHAVI	51.2
Lawrence Corey	U Wash/Hutchinson	HVTN	41.7
James Neaton	U Minnesota	INSIGHT	35.4
Paul Covington	PPD Dev. Inc.	Contract Res. Org.	33.5
Constance Benson	UC San Diego/SSS	ACTG	31.5
Sten Vermund	Vanderbilt/Family Health Int'l	HPTN, Fogarty Training	23.3
Sharon Hillier	U Pittsburgh	MTN	22.2
Brooks Jackson	Johns Hopkins/SSS	IMPAACT	19.8
Juliana McElrath	U Wash/Hutchinson	HVTN Lab	18.4
Michael Hughes	Harvard	IMPAACT Stat Ctr.	14.2
Total			**291.2**

SSS = Social and Scientific Systems Inc.; CHAVI = Center for HIV/AIDS Vaccine Immunology; for other acronyms, see Pack Mentality

Pack Mentality

Major NIH Networks (FY '07)	$M
AIDS Clinical Trial Group (ACTG)	100.8
HIV Vaccine Trials Network (HVTN)	61.7
International Maternal-Pediatric-Adolescent AIDS Clinical Trials Group (IMPAACT)	54.7
International Network for Strategic Initiatives in Global HIV Trials (INSIGHT)	36.3
HIV Prevention Trials Network (HPTN)	29.4
Microbicide Trials Network (MTN)	22.9
Total	**305.8**

Territorial Markers

Highest Impact HIV/AIDS Authors

AUTHOR	INSTITUTION	CITES	PAPERS	AVG. CITES/ PAPER
Andrew McMichael	Oxford	6955	101	68.9
Douglas Richman	UC San Diego	9934	152	65.4
Stefano Vella	ISI	6477	114	56.8
Joseph Sodroski	Harvard	7976	141	56.6
Robert Doms	U Penn	6374	113	56.4
John Moore	Weill Cornell	7163	132	54.3
Andrew Carr	U New S Wales	5814	110	52.9
David Montefiori	Duke	7694	156	49.3
Norman Letvin	Harvard	7064	161	43.9
Bruce Walker	Harvard	8700	203	42.9

Papers published 1998–2007, >100 papers


The New England Journal of Medicine

Top cited paper. Pallela *et al.*, *NEJM*, 26 March 1998, p. 853.

Highest Impact Institutions

ORGANIZATION	CITES	PAPERS	AVG. CITES/ PAPER
NIAID	56,648	1589	35.7
Northwestern	23,491	769	30.5
Harvard	115,972	4424	26.2
Duke	25,843	1000	25.8
NCI	54,947	2143	25.6
Oxford	19,952	785	25.4
Stanford	21,102	842	25.1
Washington U	13,767	570	24.2
UC San Diego	33,358	1406	23.7
U Colorado	17,547	765	22.9

Papers published 1998–2007, >500 papers

Uganda Confronts Corruption, Slowly

When allegations surfaced that Global Fund grants had been pilfered, a high-level commission promptly investigated and wrote a scathing report—that sat on shelves

The sum total of the enormous catalogue of flaws, shortcomings, *errors, mistakes, and hiccups enumerated and detailed in all the above … adds up to a humongous picture of grand managerial inefficiency and incompetence. … The great losers in this sordid story were the people of Uganda; the international donor community; and, particularly so, the new experiment in Global Fund Public-Private Sector partnership. Above all, the most poignant and pathetic losers were the millions of persons infected and affected by the three scourges of HIV/AIDS, TB and Malaria. These millions watched in pain and agony as their dire hopes were dashed to dust through the incompetence of the [money's] stewards, and the naked greed of the middlemen (the sub-recipients) of the Global funds.*

—Final report, the Judicial Commission of Inquiry into Alleged Mismanagement of Global Fund to Fight AIDS, Tuberculosis, and Malaria in Uganda.

KAMPALA, UGANDA—This blistering report landed on the desk of Ugandan President Yoweri Museveni in May 2006. The Judicial Commission of Inquiry concluded that officials, from government ministers to workers at community organizations that nominally exist to help HIV-infected people, had stolen money from the $45.3 million disbursed to Uganda from the Global Fund to Fight AIDS, Tuberculosis, and Malaria. As a result, Uganda, which had been widely praised for its exemplary response to its HIV/AIDS epidemic, has endured unprecedented public humiliation. "What is sad is this money is given to Uganda by other governments, and they're trying to help us with a huge problem," says AIDS researcher David Serwadda, who heads the School of Public Health here at Makerere University. "There's a lot of hope and trust put into us. To find that this resource has not been used to its intended purpose makes you wonder … whether we are not our own worst enemies."

Museveni appointed the judicial commission to show that Uganda would use all its muscle to root out corruption in the use of money from the Global Fund and punish wrongdoers. Headed by James Ogoola, an esteemed judge on the country's High Court who for more than 20 years worked as a legal counsel for the International Monetary Fund, the commission interviewed more than 150 witnesses over 6 months and introduced some 500 exhibits into evidence. The Ogoola commission's final 485-page report to Museveni cataloged how between 2003 and 2005 an astonishingly large cast of characters plundered five Global Fund grants in an equally astonishing array of alleged swindles.

On 30 November 2006, 6 months after Museveni received the commission's report, the Ugandan government issued a "white paper" that agreed with almost all of the findings and urged aggressive prosecution of several government officials; it also agreed that individuals and organizations should pay back at least $1.6 million.

And then nothing happened.

Not until April of this year did any follow-up to the Ogoola commission's recommendations occur, and even now, the wheels of justice appear to be spinning slowly. The government pleads guilty for the delay but says it lacked the money to carry out the prosecution. Others are far harsher. "What was the point of spending so much money on this commission of inquiry, on top of what already was embezzled, and then the government does not proceed to prosecute these individuals?" asks Beatrice Were, an HIV/AIDS advocate based in Kampala and co-founder of the National Community of Women Living With HIV/AIDS. "It does look like corruption is normal and that anyone can do what they want and walk away scot-free in this country."

Stig Barlyng, the Danish ambassador to Uganda and one of the most outspoken crusaders against corruption in the country, says the "lack of concrete follow-up" is "an embarrassment" to both Uganda and the donors. The scandal is "a litmus test" not only for how serious Uganda is about corruption, he says, but also for how well the 6-year-old Global Fund monitors recipients and reacts to wrongdoing. And this is no "victimless" white-collar crime, he adds. People were directly harmed. "When we're dealing with AIDS, it's a deadly issue. It's as simple as that."

The $1.6 million or more that was stolen from the Global Fund grants—the full amount remains unclear—might not seem like a lot, says Were, but "every coin counts" in Uganda. "If you translate it to what it means to people in a small village outside of Kampala, it's a lot." People who are affected most by this epidemic actually don't need much to transform their lives, she says. And that little was taken away.

Strong words

The Global Fund, which prides itself on rigorous monitoring and evaluation of grant recipients, did not discover the corruption in Uganda. The problem surfaced on 13 June 2005 when a whistleblower sent an e-mail to Bernard Rivers, head of Aidspan, an independent watchdog of the fund (see sidebar, p. 524). Rivers did not know the whistleblower, who worked at a Ugandan nongovernmental organization (NGO), and the first e-mail he received was all of four sentences. "It was so cryptic that I hardly knew what he was claiming, let alone whether I believed it," said Rivers. With the whistleblower's permission, Rivers passed the tip to the fund. "I was amazed when I got a reply within 2 hours, making it quite clear that Global Fund was taking it quite seriously," he says.

A few weeks after receiving the tip, the Global Fund's chief legal counsel met with the whistleblower in Kampala. Convinced that the allegations had substance, the fund asked the auditing firm PricewaterhouseCoopers to evaluate $26 million the Global Fund had disbursed through the first of five grants it had awarded to Uganda. (The fund

Dashed hopes. Mayanja Lubwama says the Global Fund theft hit home.

CREDIT: MALCOLM LINTON

The High Cost of Stolen Funds

NSANGI, UGANDA—Mayanja Lubwama, a 44-year-old HIV-infected man who lives with his wife and four of their six children in a cramped house on the outskirts of Kampala, is one of many Ugandans who were hurt by the Global Fund to Fight AIDS, Tuberculosis, and Malaria corruption case (see p. 522). "Some of us benefiting from those funds felt very sad," says Lubwama.

In many ways, Lubwama, whose wife and eldest son are also infected, is lucky: He started receiving anti-HIV drugs 8 years ago and has responded to the same regimen ever since. But during the time that the Global Fund suspended Uganda's grants, between August and November 2005, Lubwama says the clinic where he gets his anti-HIV drugs had "stock outs." He also had a job working for an HIV/AIDS nongovernmental organization that received Global Fund support; they had to let him go, and the project he worked for never restarted. "All of these projects were stopped because of the Global Fund saga," says Lubwama. And short on cash, Lubwama sometimes had to choose between paying for food for his family or transportation to a clinic to pick up his drugs.

There's no way to tally the number of people who were hurt by the corruption, but according to the latest data, at the end of 2006, 60% of the people who needed antiretroviral drugs in Uganda had yet to receive any. The Global Fund also declined to renew Uganda's grants for malaria and TB in October 2006 because of the management problems that surfaced during the corruption probe. More recently, a progress report for the main HIV/AIDS grant showed "unacceptable" performance for money disbursed between October 2006 and March 2007. "Implementation of this grant has been delayed due to the loss of momentum that occurred when all of Uganda's [Global Fund] grants were suspended," the report states.

The corruption case also forced Uganda to reorganize how it moves money from the Global Fund to various recipients. This restructuring, in turn, has slowed the country's ability to build a more sophisticated program that, for example, can routinely provide second-line treatment to people whose first-line treatment has failed. That problem now confronts Lubwama, who has started to have serious side effects from his anti-HIV drugs, including neuropathy in his feet and stomach pains, but his doctors say they don't have other drugs to offer him. "I don't have any options," he says.

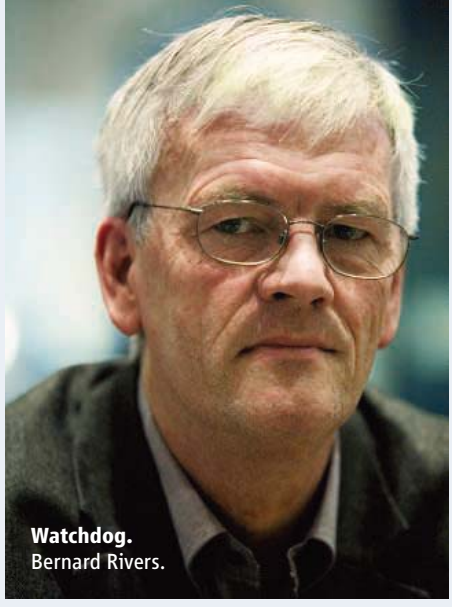
Jennifer Bakyawa, a Kampala-based journalist who now does HIV/AIDS community outreach at Makerere University, points out that the corruption harmed many people beyond those in need of treatment. Bakyawa, who has lost two half-sisters and a half-brother to HIV, says when people don't receive free anti-HIV drugs, they often turn to family and friends for financial help. If a person dies from AIDS, it's the family and friends who step in to care for his or her children. And with an adult HIV prevalence rate in the country that has ranged from 6% to 15% during the past decade, Bakyawa says, "if someone in Uganda tells you they haven't been affected by HIV/AIDS, they're lying." **–J.C.**

planned by 2010 to disburse $371 million to Uganda for all five grants, which would help the country battle HIV/AIDS, tuberculosis, and malaria.) The confidential evaluation, a copy of which *Science* obtained, identified several of the problems that the Ogoola commission later would report.

On 24 August, shortly after reading the evaluation, Brad Herbert, then the chief operating officer of the Global Fund, suspended all five grants to Uganda, allowing funding to continue only for "lifesaving treatments" and critical prevention needs such as condoms. "The Pricewaterhouse report very clearly indicated to my satisfaction that Global Fund money was not being utilized for what it was intended for," says Herbert.

Just 1 week earlier, the fund had canceled grants to Myanmar because of new restrictions imposed by the ruling junta. Uganda's

The Global Fund's Best Friend?



Watchdog. Bernard Rivers.

NAIROBI, KENYA—By Bernard Rivers's own description, he's a "loving watchdog" of the Global Fund to Fight AIDS, Tuberculosis, and Malaria, "a dog that spends much more of its time wagging its tail than barking," he adds.

Rivers and his two-person team here run Aidspan, which began publishing an electronic newsletter, the *Global Fund Observer*, shortly after the fund opened for business in 2002. The *Observer*, published every month or so and now a must-read for nearly 8000 subscribers in 170 countries, offers original analyses of Global Fund data. These include a grants-evaluation system that tracks whether disbursements are on time, news reports on Global Fund board meetings, and critical editorials about how the fund can improve. He has endorsements on his Web site from the current director of the fund, the chair of its board, and the head of UNAIDS. Rivers is "very open-minded and stubborn at the same time," says Peter van Rooijen, a former board member of the Global Fund, who now heads the International Civil Society Support in Amsterdam. "He performs his watchdog role quite eloquently." But when Rivers triggered an investigation of the Global Fund itself, its spokesperson, Jon Lidén, publicly complained. The investigation unfairly stained the credibility of the leadership, Lidén now says, noting that the probe found no serious wrongdoing.

Rivers became intrigued by the Global Fund's design soon after it formed. "Over the past 50 years, Western governments have all too often told developing countries, 'We will provide you with aid if you will support us in the Cold War, or buy our products, or follow our moral agenda, or let our nationals run your project,' " says Rivers, an economist by training. "This is an outdated and inappropriate approach, which the Global Fund model seeks to circumvent." Rivers sold his company, which provided grants-management software to foundations, and invested his own money in starting Aidspan.

With a total of four staff members (others work from England and Thailand) and an annual budget of $750,000, Aidspan finances its work with help from foundations, industry, and individuals. Rivers maintains a strict independence from the fund and takes no money from it. Although he is a critic, he says he shares the group's vision of "country-led" and "results-based" funding; he also calls it "remarkable" that in 6 years the fund has gone from $0 to giving out $3 billion annually. "Of *course*, not everything has worked perfectly from day one," he says, pointing to the recent corruption scandal, among other problems (see main text).

This past winter, Rivers interviewed more than 50 Global Fund recipients in seven African countries to examine the barriers that have prevented countries from aggressively scaling up anti-HIV drug treatment and prevention efforts. In a report published in April, he concluded that the proposal process is too "long and complex." Countries that have several grants for different diseases must contend with an "administrative burden" that makes them feel as though they have been "punished for success." The report also advocates that the fund dramatically increase assistance for strengthening health systems more broadly.

Ultimately, Rivers believes that developing countries should run Aidspan, which is one reason he moved its headquarters from New York City to Nairobi in 2007 and began hiring Kenyan staff members. "The sooner Aidspan can get to that point, the better," he says, estimating that the process will take two more years. "Founders of organizations should always get out in less than 10 years," says Rivers. "Otherwise, the organization will find it very hard to grow beyond simply being an extension of that person." But for now, as the saying goes about a happy dog, it's like he has two tails. **–J.C.**

grants were not terminated but merely put on hold, Herbert stresses, adding that he explained to a senior Ugandan official that if the government responded properly, he'd lift the suspension. "I said, 'Either you can embrace me or fight me. If you embrace me—I know that every country has corruption, my own country has corruption—what is it you will do to get the suspension lifted?' "

Herbert went to Uganda at the end of August and met with President Museveni and other officials to determine how to proceed. "There was a very serious commitment from Museveni to get to the bottom of this," says Herbert. "I think he stepped up to the plate." On 6 September, Museveni announced the formation of the Ogoola commission.

Two months later, on 10 November 2005, the Global Fund lifted the suspension, noting that Uganda had taken "significant steps" to strengthen grant management and oversight.

The Ogoola commission handed Museveni its report on 31 May 2006. Among the many alleged offenses spelled out in the report:

- Recipients billed for "ghost trips" that never occurred, inflated hotel charges, and engaged in activities to push forward a national referendum unrelated to HIV/AIDS.
- The Ministry of Health spent nearly $25,000 on medical care for a former government minister and Museveni ally.
- Another $19,832 was "borrowed" to send the minister of health on a multicountry trip to lobby for a Ugandan to win the slot as the next director-general of the World Health Organization.
- Forgeries of receipts were "rampant," and there were outright frauds, cover-ups, and kickbacks.
- The Uganda Centre for Accountability—an NGO that received more than $70,000 to monitor and evaluate other subrecipients of Global Fund money—"had absolutely no accounting capacity." (It also had the audacity to bill for fuel for a tractor, which it claimed was a vehicle used by supervisors to visit sites.)
- Nepotism led to jobs and travel grants.
- Another NGO, the National Forum of People Living with HIV/AIDS, was supposed to funnel some $160,000 to 21 other groups but ended up keeping about half of that money and billing for expenses that the commission deemed "extremely suspicious."

After receiving the commission report, Richard Feachem, then the executive director of the Global Fund, in a press release commended the Uganda government. "The openness and thoroughness with which President Yoweri Museveni addressed the Global Fund's concerns about the management of the grants it finances in Uganda has set an example for how allegations of corruption should be dealt with," wrote Feachem. Community representatives similarly were impressed.

Little action

President Museveni went even further. He passed the commission report on to his Cabinet, which issued a November 2006 white paper calling for pursuing potential refunds and criminal prosecutions

CREDIT: MALCOLM LINTON

"within 6 months." But by that deadline, May 2007, the Global Fund corruption case had disappeared from the headlines and the prosecutor's docket. "The cause of the delay was sorrily lack of funding," says Uganda's director of public prosecutions, Richard Buteera, a lead investigator in the case. An investigation this complex required help from several branches of government, Buteera says, and ordinary budgets could not cover the costs. The Cabinet did approve extra funding, but, he says, "unfortunately, it was not released in the last budget." Buteera and his colleagues had to put the case on hold.

The notion that Uganda could not immediately afford to pursue the Ogoola commission's recommendations strikes advocate Were as a flimsy excuse. She notes that the government poured tens of millions of dollars into hosting the Commonwealth Heads of Government Meeting—which the Queen of England opened—last year. "It's the commitment and the will to address corruption that [are] lacking," says Were. The country also quickly found the money to prosecute three of the same health ministers implicated in the Global Fund case who in June 2006 were charged with misusing funds from what was then known as the Global Alliance for Vaccines and Immunization. Each was arrested and is out on bail pending a trial that has been delayed because of procedural issues. (Museveni, without explanation, replaced all three of them in May 2006.)

Adolf Mwesige, the minister for general duties who chaired the Cabinet's evaluation of the Ogoola commission report, says he is not frustrated by the pace with which Uganda has followed up on its recommendations. "I'm realistic," says Mwesige. "I know the conditions in which we are operating are a little more difficult than in the West." He notes that Uganda is confronting many similar corruption cases and says that "we can't overwhelm all our institutions of prosecution." He says the government moved more quickly in the vaccine fund case because the evidence was more clear-cut. "It didn't even require a commission of inquiry."

Nor did Global Fund head Michel Kazatchkine push, telling *Science* in March 2008 that he did not see pursuing prosecution of wrongdoers as a Global Fund responsibility. "That's a Uganda process," said Kazatchkine. "This is for Uganda to fix." He claimed, too, that the fund has already recovered $700,000 of the pilfered money.

Poverty? Pshaw. Beatrice Were doubts that Uganda couldn't afford to prosecute.

CREDIT: MALCOLM LINTON

Push to prosecute

But when John Parsons joined the fund as its inspector general early this year, he put the Uganda case at the top of his priority list. In April, several members of the Global Fund's board also urged Parsons to take aggressive action. As one board member wrote Parsons, "Unless the investigations commence in the coming months, it is unlikely that the suspects can be successfully prosecuted given the lapse of time, the evidence going cold and key witnesses no longer being available, willing or reliable."

> *"It's the commitment and the will to address corruption that [are] lacking."*
>
> —Beatrice Were

That same month, Uganda's Ministry of Finance allocated 200 million Ugandan shillings (more than $125,000) to Buteera and his colleagues, about 10% of what they need but enough to get started. The U.K.'s Serious Fraud Office also offered to help. "I'm strong that we should solve this problem, take the appropriate action, get the culprits, and bring them to book," said Buteera in early May. "I wish we had the funds to do it earlier than now."

In mid-May, Parsons went to Uganda to meet with people connected with the case, and they committed to investing another 760 million shillings as of July. "My mission had an impact in unlocking political will and funds to investigate," Parsons told *Science*. "I'm very keen to encourage them to do what they should be doing and should have done some time ago." In all, he says the Ugandans agreed to investigate 373 cases and to reassess how much money is due to the fund. (Even the fund is confused about this last point: To date, Parsons says only 698 million shillings, less than $450,000, have been returned—about 65% of the amount that fund director Kazatchkine told *Science* had been recovered.) Parsons plans to update the finance and audit committee of the Global Fund in late September.

Herbert, the fund's former chief operating officer, says he expects that similar corruption has probably gone undetected with several other Global Fund grants and that there's only so much that can be done to prevent and catch thievery without causing harm. "For every 100 apples in the barrel, you probably have 10 that are bad," says Herbert. "If you put so many procedures in place that those 10 bad apples don't do anything, you might hurt the other 90. The only way I could guarantee that a dollar would not fall into corruption is to never, ever spend that dollar." **–JON COHEN**

Botswana's Success Comes at Steep Cost

HIV/AIDS has walloped this tiny country, but it has hit back hard with a pioneering treatment program. Prevention efforts have lagged, however, and could unravel the gains

FRANCISTOWN, GABORONE, AND SELEBI-PHIKWE, BOTSWANA— A few minutes before dawn on this cool June morning, 67 men at the BCL mine slip into overalls and gum boots, belt on heavy batteries that power their head lamps, and then cram into elevators large enough to hold pickup trucks. After descending 750 meters, the men trudge for 30 minutes through muggy, mud-floored tunnels that lead 135 meters deeper underground, pinning themselves against the rock walls each time a front loader hurtles by. Their lamps illuminate flecks of nickel and copper in the walls and ceiling, and later today they will harvest these valuable metals. But before the drilling and blasting starts, the men gather in a cave carved into a wall and take seats on wooden benches. As sweat pours from their faces, they join in prayer, closing with a robust "Amen."

This morning, as occurs twice a month, the prayer is followed by a "toolbox talk" about HIV/AIDS. Mines are dangerous places, but collapses and suffocation are not the only threats. Here in Selebi-Phikwe, a small town in Botswana's northeast, mining is closely linked to the spread of HIV.

About 300,000 of the 1.8 million people in Botswana have been infected with HIV, which, according to government estimates, translates to an adult prevalence in 2007 of 26.9%, among the highest in the world. Selebi-Phikwe, where BCL employs 4200 people, is one of the country's hardest hit districts. A 2003 study of BCL miners found an overall HIV prevalence of 41.3%, which jumped to 57% among 30- to 39-year-olds. "It was really staggering for us to realize this," says Montwedi Mphathi, the mine's general manager. The mine decided that it was good business to launch a comprehensive workplace program to help the infected and promote prevention.

Deep underground, the peer educator promotes the value of positive living and the merits of condoms. He holds up a flip chart that shows gruesome images of lesions from other sexually transmitted infections, urging the men to get tested if they suspect they have caught syphilis or gonorrhea. But when the educator opens the floor to questions, it's clear that the miners are more concerned about their HIV infections. "If both parties are positive and want to have a baby, what should they do?" asks one man, who wears the gold watch earned after 25 years at BCL.

Safety first. Novel prevention efforts aim to dig Botswana out.

CREDIT: MALCOLM LINTON

"Don't decide on your own," the peer educator advises. "See a medical doctor."

Like BCL, Botswana has aggressively responded to its HIV/AIDS epidemic, and this miner's sophisticated question—and the sound answer—reflect that infected people have options not available in much of sub-Saharan Africa. The government of this relatively wealthy country provides anti-HIV drugs to almost everyone in need; free medical care is widely available; and campaigns for prevention of mother-to-child transmission (PMTCT) have made great strides. Festus Mogae, who ended a 10-year term as president in April, has won international praise for his bold battle against Botswana's HIV/AIDS epidemic. Under his leadership, this tiny country formed novel partnerships with leading universities, pharmaceutical companies, foundations, and developed country governments, which have provided generous funding, state-of-the-art equipment, training, and expertise. On most fronts, says Peter Piot, head of the Joint United Nations Programme on HIV/AIDS (UNAIDS), Botswana is "doing a great job."

But progress has not come easily, and there is one glaring deficiency, says Piot: prevention. Botswana has made precious little headway slowing HIV's spread, which is transmitted here mainly through heterosexual sex. "Prevention of new infections should be priority number one, number two, and number three," says former President Mogae, who continues to chair the National AIDS Council. "We cannot treat the epidemic away."

Threatened extinction

When struggling Botswana made its transition from British rule to independence in 1966, it mainly exported cattle. But the economy exploded with the discovery of diamonds in the 1970s, and today the country is solidly middle income, akin to Russia or neighboring South Africa. An excellent highway system connects the entire country, crime is low, and the political environment is relatively stable and corruption-free.

When Mogae took office in 1998, he realized that HIV had begun to undermine many of the country's gains. "Like a war, it was taking the cream of society in terms of youth and health," says Mogae, an economist by training. Improvements in infant mortality rates, adult life

Taking it to the streets. The Ghetto Artists in Francistown play miners and their pregnant wives deciding whether to take HIV tests.

CREDIT: MALCOLM LINTON

expectancy, and other development indices disappeared.

On the eve of the international AIDS conference held in nearby Durban, South Africa, in July 2000, UNAIDS pegged Botswana's adult prevalence at 35.8%, the highest in the world. "We are threatened with extinction," Mogae said at the meeting.

"Everyone said if you can't do it in Botswana, the easiest place, you can't do it anywhere."

—Ernest Darkoh

The Durban meeting marked the beginning of a unique public-private partnership that would catalyze Botswana's drive to become the first country in sub-Saharan Africa to provide widespread access to antiretroviral drugs (ARVs): what's now called the African Comprehensive HIV/AIDS Partnerships (ACHAP). The pharmaceutical giant Merck & Co. and the Bill and Melinda Gates Foundation announced that they would each chip in $50 million of assistance over 5 years to help "reduce the spread of HIV and to significantly increase the awareness, prevention, diagnosis, and treatment of HIV/AIDS."

But the initiative had a bumpy start. Its first director, a Merck executive, clashed with government employees, who complained that he didn't understand the "culture of consultation" in Botswana. And putting together a nationwide treatment program required a massive recruitment of new professionals: A team of pro bono consultants from McKinsey & Co. estimated that the country needed staggering increases in doctors (29%), lab technicians (115%), and pharmacists (179%).

Realizing that the program was having teething pains, ACHAP's director asked one of the McKinsey consultants, Ernest Darkoh, to move to Botswana in late 2001 and help. Shortly after he arrived, Darkoh, a young American physician who also had a master's in business administration, deemed the situation "an impending disaster." Morale was poor. Leadership roles in the new program went to clinicians who had little experience as managers. Government ministries had difficulty working together; no one seemed capable of making budgetary decisions; and Darkoh had no real authority himself. "They couldn't get themselves out of the starting blocks," says Darkoh, who now lives in South Africa and heads BroadReach Healthcare.

When the government decided to start rolling out treatment in January 2002, Darkoh was convinced that they weren't ready. But he also felt that they had to succeed. "I knew what this represented to the continent; everyone said if you can't do it in Botswana, the easiest place, you can't do it anywhere," he recalls.

Darkoh's frustration mounted over the next month—Botswana, he concluded, did not deserve its reputation as an easy place to work. When he threatened to resign, the government responded by offering him a slot in the Ministry of Health (MOH) as operations manager of the program. Darkoh, then 31, took the job. "I went back to my hotel room and literally sat on my bed, saying, 'Good god, what am I going to do now?' "

The program slowly began to make progress, but it also faced intense criticism, some of which Darkoh says was unfair. The initial

Firsthand knowledge. An HIV-infected counselor at a Francistown clinic helps a patient decide whether to start ARVs.

McKinsey feasibility study projected that with staff and other resources in place, Botswana could have 19,000 people on treatment within a year. At the end of 2002, only 3200 people had begun treatment. "Despite the herculean success in getting the program on its feet, we had a big sign around our neck that this was somehow a failure," says Darkoh. "I got nasty e-mails from around the world from experts who claimed they would have done better." (McKinsey, he notes, did fail to consider that the first patients to seek treatment would have late-stage AIDS and need extra resources.)

By the time Darkoh left ACHAP in 2005, it was a trusted partner, helping Botswana provide free anti-HIV treatment and state-of-the-art monitoring to 50,000 people. "The government ARV team was functioning like an efficient machine," says Darkoh. But the success in 2005 was qualified: More than twice that number still needed treatment. The problem wasn't money—ACHAP had spent only about $55 million of the $100 million offered by the Gates Foundation and Merck—but "absorptive capacity," concluded the Gates Foundation in a 2006 report. As the report explained, "we underestimated just how hard it is to build up the systems necessary to confront HIV/AIDS across an entire country."

Not only did Botswana have a dearth of trained professionals and middle managers, the "culture of consultation" meant it had an abundance of bureaucracy. "The irony in Botswana is that part of the problem is the inheritance of the British civil servant's approach," says Michael Reich, a political scientist at Harvard University who co-authored an analysis of the early ACHAP efforts in the July 2006 issue of *Social Science & Medicine*. "Some people say they're more British than the British."

Stopping spread

As of March 2008, 97,265 people at 32 dedicated ARV sites around Botswana were receiving anti-HIV drugs—nearly 90% of those most in need. And the drugs are working. Researchers from Botswana's MOH and Harvard jointly run an AIDS institute in Gaborone, and in 2007, they reported that potent cocktails of anti-HIV drugs had powerfully suppressed HIV for more than 2 years in the patients they studied, and only 2% developed drug-resistant mutations. "The era of ARVs has saved the nation," says epidemiologist Khumo Seipone, head of HIV/AIDS prevention and care at MOH.

The Botswana government has financed the bulk of its HIV/AIDS response—it spent about $130 million last year alone—and it has also received more than $250 million from the U.S. President's Emergency Plan for AIDS Relief since 2004. But many say ACHAP's investment had far-reaching impact. "ACHAP did not just support Botswana; it galvanized the global commitment to treatment," says Joy Phumaphi, the minister of health from 1999 to 2003 and now a vice president at the World Bank in Washington, D.C.

Alarming point. Former President Mogae warned that HIV could wipe Botswana from the map.

CREDITS (TOP TO BOTTOM): MALCOLM LINTON

Merck and the Gates Foundation extended ACHAP through 2009, and when it ends, the program will have spent $106 million. They are considering launching a second phase to help Botswana address the elephant in the room: prevention. "Clearly there's a need for a catalyst in the area of prevention," says Themba Moeti, ACHAP's managing director.

Botswana has encouraged people to have fewer partners, delay the age of first sex, increase their condom use, and adopt other behavioral changes, but none has made dramatic progress. The PMTCT program, which offers anti-HIV drugs to infected pregnant women and newborns to protect the babies, has had trouble too since its start in 1999, even with substantial support from ACHAP and the U.S. Centers for Disease Control and Prevention (CDC). In 2003, only 47% of women who visited a hospital in Francistown, the country's second-largest city, knew their HIV status, and of those who tested positive, 71% did not take ARVs to prevent transmission, reported CDC pediatrician Tracy Creek and colleagues in the 1 May 2007 issue of the *Journal of Acquired Immune Deficiency Syndromes*. Many pregnant women were simply not volunteering to take HIV tests, and those who did often did not act on the results.

But PMTCT also shows that a struggling prevention effort can, like the treatment program, find its footing. In a New Year message at the end of 2003, Mogae announced a more aggressive policy that would alter PMTCT's fate: Bucking the international standard of "voluntary counseling and testing," clinics would routinely test people for HIV unless they specifically opted out. According to government figures, in March 2007, 83% of pregnant women took an HIV test, and 89.9% of those who tested positive chose to take ARVs. Transmission to babies has dropped from 40% in 2001 to 4% last year. "Routine testing has been a smashing success," says Creek.

Yet outside of PMTCT, Botswana has little to show for its prevention interventions. When prevention efforts work, new infection rates should drop. The scant evidence here suggests that at best, says MOH's Seipone, incidence has stabilized and may have dropped in pregnant women between 2005 and 2007. But prevalence remains astonishingly high among pregnant women: Reports from antenatal clinics show a prevalence of 33.7% in 2007. One of the key reasons for the failure of prevention efforts, says former health minister Phumaphi, "is we are not addressing the drivers of the epidemics," by which she primarily means heterosexual behaviors. She worries, too, that the success with ARVs has increased complacency about becoming infected.

Botswana set the goal of "zero new infections by 2016" and last December issued an intensified prevention plan. At the top of the list is reducing concurrent sexual partnerships. In Botswana tradition, men have a large house for their main wives and small houses for their mistresses. Women often also have more than one partner, who some jokingly refer to as their ministers of cell phone, rent, food, or jewelry. Many Botswanans have highly mobile lifestyles: Cars are abundant, and on weekends, urban workers—say, miners in Selebi-Phikwe or professionals in Gaborone—often return to their rural villages, without their city partners.

The prevention plan also urges Botswana to better communicate the dangers of young women having sex with older men (who have a high prevalence), heavy alcohol use, stigma, and inconsistent condom use. And it faults the country for having "often ignored" the role of sex workers, who do a busy trade in mining towns and on major transport routes. Fewer than 20% of men in Botswana are circumcised, and many expect that the government will soon endorse this proven prevention option.

Precisely how Botswana will scale up these efforts remains an unknown. Phumaphi urges her country to rely on evidence-based strategies. "We have to be much more methodical," she says.

Civil discourse. A community group hands out female and male condoms at a shebeen, a homebrew pub.

Daniel Motsatsing, head of the Botswana Network of AIDS Service Organizations (BONASO), thinks prevention efforts should rely more heavily on community-based organizations. Botswana's generally strong government, he contends, gives people things "on a silver platter," unintentionally squelching grass-roots efforts. He also faults the government for losing more than half of an $18.6 million grant from the Global Fund to Fight AIDS, Tuberculosis, and Malaria that would have supported prevention work done by some of BONASO's 160 members. The fund ended the grant because the government's National AIDS Coordinating Agency (NACA) failed to file a required report.

> *"We are fast approaching a situation where we cannot afford what we are doing even now."*
> —Festus Mogae

Unless prevention efforts make more headway, Mogae says a crisis "is bound to come" as ever more people seek treatment and stretch resources to the limit. "We are fast approaching a situation where we cannot afford what we are doing even now," says Mogae. NACA official Monica Tselayakgosi says the country plans to apply for Global Fund money again, is attempting to purchase ARVs more cheaply, and is even considering charging some people for treatment. "The response to the epidemic is not sustainable," she says. "We need to sit back and really take a hard look at what it is that we really can afford."

–JON COHEN

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PERSPECTIVE

HIV Vaccine Research: The Way Forward

Anthony S. Fauci,[1]* Margaret I. Johnston,[1] Carl W. Dieffenbach,[1] Dennis R. Burton,[2] Scott M. Hammer,[3] James A. Hoxie,[4] Malcolm Martin,[1] Julie Overbaugh,[5] David I. Watkins,[6] Adel Mahmoud,[7] Warner C. Greene[8]

The need to broaden research directed at answering fundamental questions in HIV vaccine discovery through laboratory, nonhuman primate (NHP), and clinical research has recently been emphasized. In addition, the importance of attracting and retaining young researchers, developing better NHP models, and more closely linking NHP and clinical research is being stressed. In an era of a level budget for biomedical research at the U.S. National Institutes of Health (NIH), HIV/AIDS vaccine research efforts will need to be carefully prioritized such that resources to energize HIV vaccine discovery can be identified. This article summarizes progress and challenges in HIV vaccine research, the priorities arising from a recent summit at NIAID, and the actions needed, some already under way, to address those priorities.

With more than 6500 new infections daily, HIV has assumed the dubious distinction of being one of the most catastrophic pandemics to confront mankind. Although the search for an HIV vaccine remains among the highest public health priorities, the identification of a preventive HIV vaccine has thus far eluded the biomedical research community, mainly because of the significant scientific obstacles presented by the virus (*1*).

A logical first approach to developing an HIV vaccine was to investigate purified recombinant forms of gp120, the outer envelope protein of HIV, as vaccine candidates. Unfortunately, these products failed to protect volunteers in two phase 3 efficacy trials (*2*, *3*), probably because the vaccine failed to induce antibodies that neutralize a broad array of primary isolates of HIV. The development of immunogens that elicit such broadly neutralizing antibodies remains a high priority research goal; their current absence from our vaccine armamentarium represents a major stumbling block in the development of an effective HIV vaccine (*1*, *4*).

As research on the natural history of HIV disease progressed, a greater appreciation of the role of T cells in the control of HIV disease progression evolved (*5*, *6*). The immune response elicited by a successful vaccine likely will require both antibodies and T cells that recognize diverse strains of HIV and that reach the site of infection very quickly before infection becomes irreversibly established (*7*, *8*). Given the hurdles of eliciting broad neutralizing antibody responses, the focus turned to evaluating whether a T cell vaccine that reduces viral replication after infection might serve as an intermediate step until immunogens that additionally induce broadly functional antibodies that block acquisition are identified. The benefits of such control could include a slower rate of disease progression and/or reduced transmission of HIV from the infected vaccinee to his/her partner. Indeed, several nonhuman primate (NHP) protection studies demonstrated that vaccine candidates that elicited T cell responses enabled animals to better control viral replication after challenge with a pathogenic virus (*9*–*15*). However, the inclusion of envelope in some of these vaccines, which leads to antibody induction, and the use of challenge strains that were homologous to the vaccine inserts suggest that most of these studies were not a stringent test of the T cell vaccine concept (*16*).

The MRKAd5 HIV-1 Gag/Pol/Nef candidate vaccine advanced to a phase 2b test-of-concept trial known as STEP, conducted by Merck & Co., Inc., and the HIV Vaccine Trials Network (HVTN). The vaccine neither prevented infection nor had an impact on early plasma virus levels in those who received the vaccine compared with the placebo recipients (*17*). In addition, a completely unexpected observation emerged in the STEP trial. Although a strict statistical analysis could not be performed because the data were analyzed in a post hoc manner, there was a trend toward a greater number of vaccine recipients infected, compared with the placebo recipients. Those who entered the trial with prior immunity to the viral vector [adenovirus serotype 5 (Ad5)], who were also uncircumcised, appeared at increased risk of HIV infection if they received vaccine rather than placebo (*18*). Those with only one of these risk factors (uncircumcised or prior Ad5 immunity) appeared to exhibit intermediate risk, whereas there was no evident increase in risk among those with neither cofactor. The conclusions from this trial remain tentative given the small numbers of infections observed and the post hoc nature of the analyses.

The STEP trial results pointed to two critical areas for future research. First, did the STEP trial disprove the T cell vaccine concept, or was this a failure of the specific product, perhaps because this particular vaccine candidate did not induce immune responses of sufficient quality or quantity? For example, volunteers who received the MRKAd5 HIV-1 Gag/Pol/Nef vaccine mounted T cell responses to three to five epitopes on average. Perhaps that was insufficient to control the incoming virus. Other qualities of the cellular immune response (such as the balance between HIV-specific $CD4^+$ T cell and $CD8^+$ T cell responses, or the polyfunctionality, proliferative capacity, specificity, avidity, and the location or kinetics) may also prove important and remain to be examined.

Researchers are now utilizing STEP specimens to explore why this vaccine failed (*19*). Examining the genomic sequences of infecting HIV strains will demonstrate whether immunization resulted in early immunologic pressure on the incoming virus and may suggest which HIV genes or epitopes should be included in subsequent vaccines. Sequence information will also help elucidate whether infections clustered in social networks at certain trial sites.

The second critical research area pertains to a biological basis for the enhanced acquisition observed in certain subsets of volunteers. HIV cases did not appear to cluster around vaccination times, which suggests that the volunteers did not have enhanced susceptibility to HIV infection immediately after receiving the vaccine (*18*). Also, no differences in activated circulating T cells between vaccine and placebo recipients have been found (*20*). Additional studies with mucosal and biopsy specimens will be required to explore whether activation of cells at the mucosal sites were different between vaccine and placebo recipients. Whole-genome studies may reveal associations between host genetic background, baseline Ad5 titer, and HIV acquisition. Targeted studies are under way to determine whether a relation exists between human lymphocyte antigen (HLA) type or KIR (killer cell immunoglobulin-like receptor) genotypes and HIV acquisition and immune responses. Investigators are also working to determine whether the Ad5 vaccine elicited T cell or antibody-mediated responses that could have enhanced HIV acquisition.

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Table 1. Highest research priorities identified at the NIAID HIV Vaccine Summit, March 2008.

- Further define the first events leading to HIV and SIV's entering the gut-associated lymphoid tissue
- Determine the rate and mechanisms by which immune cells are mobilized to the site of infection and whether innate responses can alter the course of infection
- Characterize the cellular and humoral immune responses needed to control viral replication through modulation and/or elimination of specific cell subsets in the SIV model and studies of HIV-infected populations
- Determine the three-dimensional structure of HIV envelope trimer
- Determine why broadly neutralizing antibodies are uncommon and how they can be elicited
- Define the specificities of antibodies that neutralize diverse primary isolates
- Develop more relevant animal models (and challenge viruses) to explore protection or enhancement of infection or disease, especially heterologous challenge models
- Determine why SIV is apathogenic in some NHP species
- Identify correlates of vaccine-induced immune protection, especially the mechanisms whereby nonpathogenic (e.g., attenuated) SIVs prevent infection by pathogenic virus

Additional information on the summit, including the Webcast, can be found at the URL in *(21)*.

Although the vaccine in the STEP trial failed to show efficacy, the trial unequivocally demonstrated that the current simian-human immunodeficiency virus (SHIV) NHP challenge model is not appropriate for evaluating T cell vaccines; that the SIV NHP challenge model is more predictive; that immunity to vectors, including at the tissue level, should be evaluated in future clinical studies; and that this smaller efficacy trial design can yield valuable information to help guide future efforts.

After the disappointing results in the STEP study, the National Institute of Allergy and Infectious Diseases (NIAID) held a scientific summit in March 2008 (*21*, *22*) to solicit input on how best to reinvigorate and advance the field of HIV vaccine discovery research. The need to broaden research directed at answering fundamental questions in HIV vaccine discovery through laboratory, NHP, and clinical research was emphasized (Table 1). In addition, the importance of attracting and retaining young researchers, developing better NHP models, and more closely linking NHP models and clinical research was stressed.

Shortly after the summit, NIAID solicited additional input on how HIV vaccine discovery should be broadened and supported (*23*). Information from the summit and this solicitation will be used to help craft broad initiatives to stimulate HIV vaccine discovery research in 2009 and beyond.

The summit provided no clear consensus on whether a vaccine should demonstrate efficacy in a NHP model of AIDS as a criterion for entering clinical trials (the "gatekeeper" role). Yet it was clear that expansion of NHP vaccine studies could contribute to vaccine discovery research (Table 1). NIAID is partnering with the National Center for Research Resources, the directors of the U.S. National Institutes of Health Regional Primate Centers, and others to examine how best to ensure the availability of sufficient numbers of NHPs and the appropriate infrastructure to support vaccine discovery research. Expansion of center capabilities is being explored. A workshop to further explore research needs and approaches in more detail is planned for the fall of 2008.

One area in particular that requires attention is the design of parallel NHP and clinical studies so that results from these studies are more directly comparable. The HVTN in collaboration with NHP researchers is launching a pilot program to support the exchange of researchers, including young investigators, between the clinic and NHP facilities so that common questions in HIV vaccine discovery can be identified and addressed using common tools. For example, does a specific vaccine such as Ad5 induce the same immune responses and degree of cell activation at mucosal sites in NHPs and in humans? Can the use of heterologous gene inserts increase the breadth of immune responses? Does electroporation of DNA alter the qualitative or quantitative nature of induced immune responses? Data from human and NHP studies that are more directly comparable will help identify and validate the most predictive NHP model(s).

Some empiric evaluation of candidates in humans that appear most promising in NHP studies should continue, with cost-benefit carefully evaluated. One needed change is the expansion of immune-monitoring tools to make such assessments. Elispot assays and intracellular cytokine analysis should no longer be the only tools used to evaluate immunogenicity. The development and validation of additional assays that measure proliferative capacity, mucosal recruitment, cytotoxic capacity, or other immune functions may provide a more robust indication of functional antiviral activity.

NIAID, with input from the extramural community, will support test-of-concept trials if a candidate vaccine is considerably improved and has reasonable potential for moving the field forward relative to candidates that preceded it in the clinical pipeline. Thus, the bar that a candidate vaccine needs to pass will be raised on the basis of accumulated knowledge from prior trials, NHP studies, and fundamental research.

The shift in research focus to less product evaluation and more vaccine discovery research will require a more nimble, robust, expandable (and contractible) clinical research infrastructure, which will be achieved through linking funding of sites to clinical research activity that is ongoing and planned for each site. It will also require new ideas. NIAID leadership has been working to help new investigators obtain their first grants. The broad HIV/AIDS innovation grant program often serves as an entry point for new investigators who lack the preliminary data required to successfully compete for an R01 grant. In addition, NIAID's current policy for unsolicited grant applications favors new investigators. NIAID is committed to exploring new avenues to attract new and young investigators into this area and maintain their involvement.

Unfortunately, the need to focus additional resources on HIV vaccine discovery comes at a time when the NIH budget remains flat. When the biomedical research and development price index is considered, the purchasing power of research dollars has decreased by >13% since 2003. In the immediate future, all current HIV product development activities may feel the effect in order to yield the funds necessary for new initiatives aimed at stimulating HIV vaccine discovery research. Underutilized HIV vaccine clinical trial sites will be encouraged to participate in other types of prevention or treatment research. Funds from contracts that do not meet milestones and unexpended balances from initiatives that do not attract high-quality applications will be redirected. Certain development contracts will either be discontinued or opened to new competitors less frequently. Should growth in the NIH budget be reinstated in future years, one of the highest priorities will be to target those additional resources to HIV vaccine programs, particularly vaccine discovery research.

Given the extraordinary genetic diversity of HIV, the many features of the envelope glycoprotein that shield the virus from antibody-mediated neutralization, and the speed at which viral replica-

tion occurs and latency is established, design of a vaccine that blocks HIV infection will require enormous intellectual leaps beyond present day knowledge. Despite the obstacles that HIV presents to vaccine researchers, the historic success of vaccines argues that HIV vaccine research must be continued and accelerated. There should be no doubt about our commitment in this regard. Furthermore, pursuit of new avenues and cross-fertilization from such fields as genetics, structural biology, systems biology, cell biology, and peptide chemistry (among others) could yield new approaches for tackling these major obstacles and generate knowledge useful in vaccine design and evaluation.

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10.1126/science.1161000

PERSPECTIVE

Whither or Wither Microbicides?

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After disappointing results from all efficacy trials conducted to date, the field of microbicides research now faces substantial challenges. Poor coordination among interested parties and the choice of nonvalidated scientific targets for phase III studies have hampered progress and created mistrust about the use of microbicides as a method to prevent HIV-1 sexual transmission. Although new promising strategies are available, there will need to be serious reappraisals of how decisions are made to advance the next generations of candidates into clinical trials, and the use of appropriate animal models in this process will be critical.

The vaginal microbicide field faces yet another of its all-too-frequent crises after the outcome of the Carraguard efficacy trial, conducted by the Population Council in South Africa. This compound, a sulfated polysaccharide (polyanion), failed to demonstrate efficacy against HIV-1 vaginal transmission.

Almost simultaneously, the U.K. Microbicide Development Program reported that the high-dose arm of the efficacy trial of another polyanion, PRO-2000, would be terminated immediately because there was no hope for demonstrating efficacy (*1*). It is a sign of the state of the field that there were sighs of relief when it became clear that Carraguard had not enhanced HIV-1 transmission rates, for this was the apparent outcome of the efficacy trial of Ushercell (cellulose sulfate), yet another polyanion, last year (*2*). With enhanced transmission occurring in the first ever microbicide efficacy trial, that of the detergent nonoxynol-9 (*3*), and probably at one of the trial sites of another detergent, Savvy (*4*), the track record of microbicide products in large-scale trials has been extremely poor. The failure of polyanions is not surprising because these compounds have limited potency in vitro, particularly against the most commonly transmitted strains of HIV-1, those that use the chemokine receptor CCR5 to enter cells (*5*, *6*). Moreover, evidence is now emerging that cellulose sulfate can enhance HIV-1 infection in vitro, particularly of CCR5-using viruses (*7*). Because similar observations of polyanion-mediated enhancement of such viruses, both in vitro and in vivo, were made 15 to 20 years ago (*8*, *9*), the subsequent testing of the polyanions in thousands of women raises concerns about the preclinical research that was performed on these microbicide candidates. Yet another detergent, sodium lauryl sulfate, is still being evaluated; the rationale for continuing this study is unclear. Questions must now be asked about the past and future directions of the microbicide field; the answers should help to frame the next phase of microbicide development.

Why were detergents and polyanions selected for efficacy trials? The simple, and probably correct, answer is that decisions were based on the belief that preventing HIV-1 sexual transmission would be much easier to accomplish than turned out to be the case. Detergents disrupt HIV-1 efficiently in the test tube, and the polyanions have at least some antiviral activity in vitro (*3*, *5*, *6*). Moreover, these compounds were cheap, available, and thought to be safe on the basis of in vitro studies. Phase 1 trials also revealed no major safety problems, although inflammation was observed in early tests of nonoxynol-9 (*3*, *10*, *11*). Given the need to generate "momentum," and the lack, several years ago, of alternatives, key decision-makers in the microbicide field presumably believed that these products should be fast-tracked. Duplication of effort was an inevitable consequence when multiple funding agencies or institutions each felt the need to adopt its own polyanion candidate. Rather than comparing the different products in an

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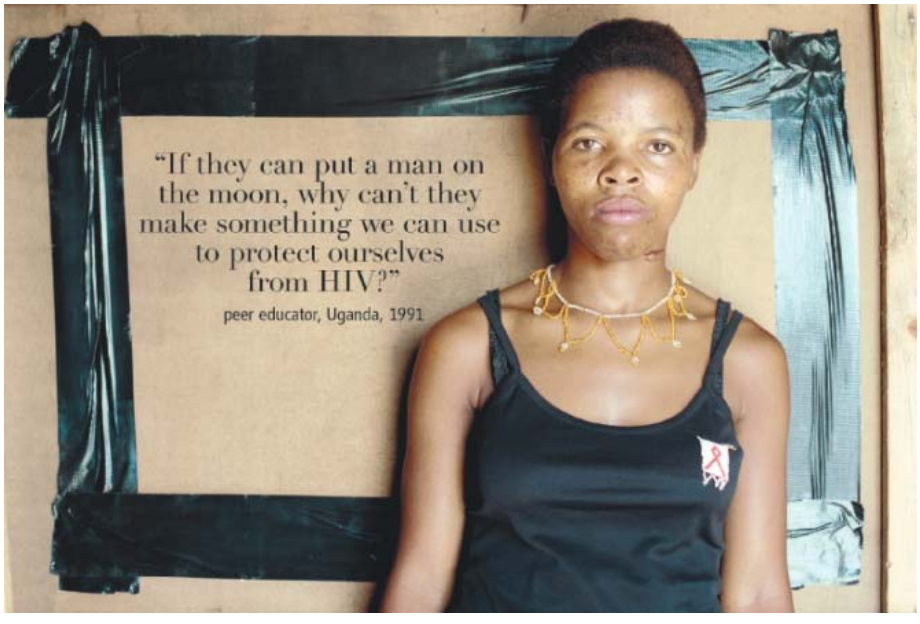
animal model, identifying "the best," and coalescing behind a consensus proof-of-concept candidate, the various agencies entered at least five such compounds into phase II human trials, with three advancing to phase III. Perhaps a test of concept efficacy trial of a single polyanion might have been scientifically valid, but what happened was, and remains, difficult to justify. The competitive spirit is important in product development, but it can (and, in the case of the polyanion-based microbicides, did) cause an unacceptable waste of resources, both human and financial.

Reverse transcriptase inhibitor–based microbicides: The next generation. The next great hope of the microbicide field is that a reverse transcriptase inhibitor (RTI)–based product might work. This concept is not without scientific merit, because the RTIs are generally potent, broadly active inhibitors both in vitro and in vivo (*12*, *13*). Conducting an efficacy trial of an RTI-based microbicide seems justified, although we will raise some caveats below. Unfortunately, the structural problems in the microbicide field could reprise the errors of the past unless they are now resolved. Thus, there may not be a single efficacy trial, or even a pair of trials, of an RTI-based microbicide; there could be as many as four. The U.S. National Institutes of Health (NIH), the International Partnership for Microbicides (IPM), the Contraceptive Research and Development Agency, and the Population Council are all competing in this area. Rather than "The Great RTI Race," there needs to be a thorough, truly open and independent assessment of whether there should be large-scale testing of any RTI-based microbicide and, if so, a determination of which compound(s) should move forward and how many trials are needed to reach a conclusion. We recognize that there is merit in evaluating multiple RTIs at the preclinical stage and in small-scale human trials, particularly with the aim of identifying the most appropriate strategy for real-world use. For example, different compounds may have different physicochemical properties that facilitate formulation, something that could be assessed in nonhuman primates and in early-stage human trials. However, we believe there would have to be truly compelling, science-based reasons to advance more than one of these compounds into efficacy trials.



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Currently, there is at best limited evidence from monkey models that topically applied RTIs can consistently protect from vaginal transmission, and most of the leading candidates have never been tested. A more substantial concern is that using RTIs for prevention might increase the spread of HIV-1 resistance, either because of their use by women who do not know their infection status, or because they select for the transmission of naturally resistant variants (*14*). There is precedence for resistance selection based on the use of nevirapine, a non-nucleoside RTI, to prevent mother-to-child transmission (*15*). What is uncertain, however, is the severity of the problem, which might in practice be minor and acceptable. The outcome may depend on whether topically applied RTIs can enter the circulation at levels high enough to drive resistance. This issue needs to be studied carefully in both nonhuman primates and humans before large-scale clinical trials are commenced. Conversely, the generation of RTI-resistant viruses during therapy, an increasing problem now in Africa, could compromise the use of RTIs for prevention. There are, therefore, some risks associated with using RTI-based microbicides in any geographical area where broadly similar compounds form the mainstay of therapy (*15*).

Alternative strategies. Rather than gambling so many resources on testing multiple different RTIs, all of which have the same basic mechanism and viral target, the microbicide field should expand its focus to test a variety of molecules that attack HIV-1 at different points in its replication cycle. These include a new generation of antiretrovirals, the fusion or entry inhibitors that have already been proven to consistently and robustly protect macaques from vaginal transmission under highly stringent conditions (*12*, *16*). Some of these compounds appear to have a high resistance barrier in vitro, and none is yet being used to treat HIV-1 infection in the developing countries where a microbicide is most needed.

Agreement could therefore be reached (at least in theory) between prevention- and treatment-based organizations and companies to use entry inhibitors only for prevention in the developing world, with RTIs and protease inhibitors being reserved for therapy. As orally administered entry inhibitors do not yet appear to have any clinical advantages over other classes of drugs (*17*, *18*), concerns about fair access to a diversity of antiretroviral agents may be trumped by the importance of preserving a plausible strategy for preventing infection. A similar agreement could be reached about integrase inhibitors, if these highly promising new compounds also prove to be useful as microbicides when tested in the macaque models. We are conscious, however, of the need to preserve some new drug classes for treatment of infection with multidrug-resistant viruses, because their emergence in the developing world must be anticipated. Moreover, the problems of resistance development during microbicide usage will apply to all classes of antiretroviral drugs, not just RTIs. These complex issues of prevention versus treatment priorities will require serious and informed discussions in the near future. Right now, these debates are not being held, with far-reaching decisions being made on an ad hoc basis. Persuading the pharmaceutical industry to restrict some of its drugs for HIV-1 prevention will not be easy, but some industry leaders have shown considerable vision in the past and may do so again given what is at stake.

An expanded role for nonhuman primate models. A critical issue for microbicides is the extent to which the nonhuman primate models have been ignored. This unfortunate situation reflects an attitude that only data derived from human trials are meaningful. The often-expressed fear is that a failed monkey experiment might lead to the rejection of a concept that could work in humans, but there also appears to be a reluctance to deal with inconvenient data that suggest a prominent microbicide strategy probably will not work in the real world. Any microbicide candidate being considered for human trials must

demonstrate proof of both safety and efficacy in a macaque model, something that would be standard procedure in any product development program run by most major pharmaceutical companies. There are, at present, uncertainties about which particular nonhuman primate model is the best for testing prevention strategies, but until the potential value of these models becomes more widely accepted, the incentive and funding required to identify a consensus model will remain suboptimal. Success in a macaque model does not, of course, guarantee that the compound(s) will protect women from the far more daunting challenge posed by diverse HIV-1 strains, but failure to protect monkeys should be regarded as a serious sign that the compound lacks potency. Nonhuman primate challenge protocols must also be developed for assessing whether microbicide candidates might enhance susceptibility to infection. The development and widespread adoption of standardized preclinical testing programs is also needed. The perceived need to conduct large-scale trials should be replaced by the real need to test only the best agents and answer the most important questions, even if this means a reduction in the size and scope of the existing clinical trials program and the involvement of fewer funding agencies.

Combination microbicides. The microbicide field is still rooted in the "monotherapy" era, in that almost all planned or completed trials involve testing a single active compound. Anyone proposing to use monotherapy to treat HIV-1 infection would not be taken seriously nowadays, and the concept of multivalent vaccines is widely accepted. Yet the microbicide field continues to hope that a single compound will suffice to protect against the vast array of HIV-1 sequence variants that now circulate and are being sexually transmitted. This attitude seems incautiously optimistic. We recognize that logistical, practical, financial, and, perhaps above all, regulatory issues presently hinder the development of combination microbicides (*19*). Some of the regulatory concerns relate to the potential for adverse events when active agents that are individually safe are combined; this scenario seems unlikely with the newer antiretrovirals. There are also considerable scientific challenges in formulating more than one active agent in a single product. Nonetheless, given the overarching need for efficacy against diverse viral populations, these problems should now be tackled head on at the scientific, product development, and (in the case of FDA's outdated regulations) political levels.

We do not suggest that development of the more plausible, highly active single-agent microbicides should be delayed; we do, however, urge that all obstacles to the evaluation of combination products should be addressed now, and not just if and when single-agent products fail. We should also consider the possibility that an effective HIV-1 prevention program might require microbicides, vaccines, and/or pre-exposure prophylaxis, as well as behavioral science interventions, to be used together, not separately. The different arms of prevention science should cooperate, not compete as they do at present.

The need to simplify microbicide usage. One useful outcome of the Carraguard efficacy trial was an indication of what had long been suspected: that women actually use microbicides much less frequently than they report doing (44% compared to 96%) (*20*). But even a potent microbicide will be ineffective if it is not used consistently and correctly. This appears to be the case with products that must be used immediately before intercourse. Until more effective microbicide regimens are developed, potential solutions to the serious problem of poor adherence are formulations that need only be applied once daily (e.g., first thing in the morning) or sustained delivery devices such as inhibitor-loaded, vaginally inserted rings that gradually release active compounds for prolonged periods (*21*). These technologies are now being developed, notably by the IPM. In the meantime, is it worth further tests of formulations that women do not use often enough, particularly when evidence is lacking of their ability to provide durable protection in nonhuman primate models?

Is an effective microbicide possible? Several candidate microbicides can completely protect nonhuman primates from vaginal infection, which serves as a valuable proof of the overall concept (*12*, *16*). The key problem areas seem now to be based in the practical issues of product development. A lack of expertise in this area might be the single biggest obstacle to success. It is a truism that pharmaceutical companies develop pharmaceutical products; nongovernmental organizations, academic scientists, and federal agencies rarely have the necessary experience. No major company has become directly involved in microbicide development, an important gap that may never be filled. One reason for the absence of the corporate sector relates to liability protection; solutions, including government-supported insurance, that apply to vaccines should be applied to other areas of prevention, including microbicides. In the absence of industry involvement, the nonprofit sector and various competing arms of the federal government have attempted to build or finance expertise in product development. This strategy is not working consistently well at present. It is arguable that NIH should now focus on its traditional role of supporting scientific discovery and that other federal agencies such as the U.S. Agency for International Development should reconsider whether their involvement in microbicide research is helpful or just adds to the competition and confusion that now prevail. Whatever structure can be put in place for the nonprofit sector and federal agencies to work together to develop a microbicide product, the same hardheaded attitudes that prevail in the pharmaceutical industry must be adopted—wishful thinking should not be tolerated.

We believe it is time for some serious soul-searching within the microbicide field, with a substantial revision of its management, better and more accountable procedures for coordinating its major programs, and more attention paid to the underlying science. It will not be straightforward to coordinate and simplify the competing activities of multiple agencies, but if we concede failure before we even try, the problems of the past will persist and we will collectively fail to produce an effective microbicide. This would be a tragedy.

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10.1126/science.1160355

A Quantitative Link Between Recycling and Osmium Isotopes

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Formation, subduction, and incomplete mixing of oceanic crust produces chemical and isotopic heterogeneity in Earth's mantle (*1*, *2*). The signature of these processes in the mantle over time and the importance of recycling in explaining the origins and compositions of volcanic rocks remain major questions. Recently, osmium isotopes have proved to be an important tracer (*3*, *4*). Basaltic ocean crust has much higher Re/Os ratios than mantle peridotite, from which it is derived through partial melting, because osmium remains mostly in the solid (mantle residue), whereas Re preferentially enters the melt. The decay of long-lived ^{187}Re to stable ^{187}Os therefore generates increased ^{187}Os/^{188}Os ratios in such crustal rocks. Elevated ^{187}Os/^{188}Os values have been recently found in Icelandic basalts, where they are correlated with the relative abundances of other isotopes (*5*, *6*). These data support the notion that the mantle source of Icelandic basalts contains oceanic crust that has been recycled by subduction and mantle convection (*7*). However, it has been difficult to assess this model because independent estimates of the proportions of components and their Os isotopic compositions were lacking.

Sobolev *et al.* (*8*, *9*) recently proposed that the abundances of Mn and Ni in early-formed olivine crystals in oceanic basalts such as Hawaii and Iceland can provide these estimates. The method makes use of fundamental differences in chemical composition, mineralogy, and melting behavior between recycled (oceanic) crust and mantle. Whereas mantle peridotite invariably contains large proportions of olivine, recycled (eclogitic) crust reacts with the surrounding peridotite and forms an olivine-free hybrid, pyroxenite (*8*). A similar hybrid component, a mixture of ancient recycled crust and peridotites, was also proposed on the basis of Os-He isotope relationships in Icelandic picrites (*6*). Because olivine and pyroxene partition Ni and Mn differently (olivine prefers Ni and pyroxene, Mn), Ni and Mn abundances in equilibrium melts formed from these contrasting lithologies will retain the memory of their respective source compositions. When a source contains both peridotite and pyroxenite, both lithologies contribute to the resulting melt, and their proportions can be reconstructed by using Mn/Fe or Ni/(Mg/Fe) ratios in olivine phenocrysts formed from these hybrid melts.

We combined data for averaged compositions of olivine phenocrysts for olivine-rich lavas and bulk rock ^{187}Os/^{188}Os ratios from Iceland to obtain the proportion of pyroxenite-derived melt by both Ni excesses and Mn deficits in olivine (Fig. 1). These parameters indeed show strong linear correlations, and this permits quantitative assessment of the end-member isotope ratios: peridotitic mantle (X_{px} = 0) and pyroxenite from recycled crust (X_{px} = 1). The best correlation based on Ni (Fig. 1A) yields a (peridotitic) intercept of ^{187}Os/^{188}Os = 0.126 ± 0.002 (2σ), similar to estimates of present-day oceanic mantle ^{187}Os/^{188}Os = 0.125 (*4*). The calculated isotopic composition of pyroxenite is ^{187}Os/^{188}Os = 0.140 to 0.155. The analogous correlation based on Mn (Fig. 1B) is consistent with this result, although the scatter is slightly greater. These values can be modeled as 1.1- to 1.8-billion-year-old oceanic crust reacted with present-day oceanic peridotite shortly before final melting (*10*). The calculated age range for recycled component in Icelandic mantle is consistent with similar age estimations from Pb isotopes (*11*) and Os and He isotope relationships (*6*). The obtained isotopic compositions of the peridotitic and pyroxenitic components for Iceland also support independently "olivine-based" estimates of source proportions (*9*), as well as the qualitative idea of the presence of ancient recycled materials in Icelandic mantle sources (*5*–*7*, *11*).

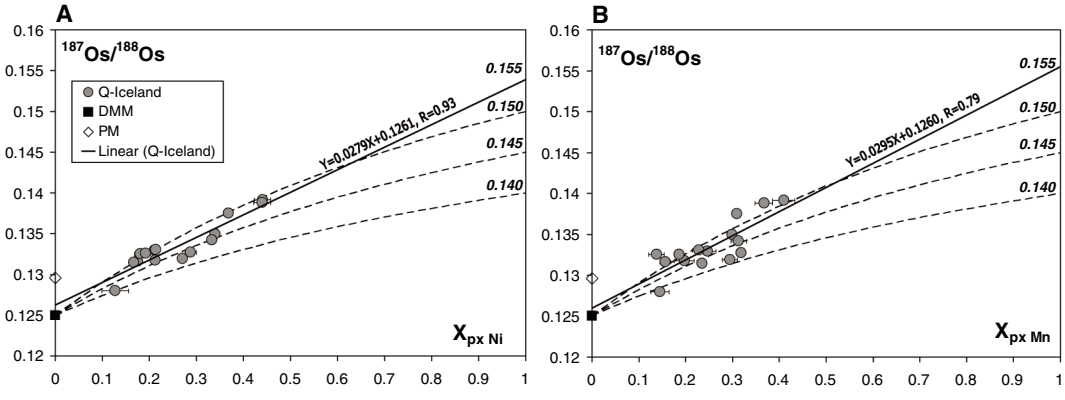

Fig. 1. (**A** and **B**) Measured bulk rock ^{187}Os/^{188}Os versus estimated proportion of pyroxenite derived melt (X_{px}) (table S1). X_{px} is defined as a following linear functions of average olivine composition (*10*): $X_{px\ Ni}$ = 10.54 × NiO/(MgO/FeO) − 0.4368; $X_{px\ Mn}$ = 3.483 − 207.139 × (Mn/Fe). Solid lines represent linear regression with parameters shown. DMM and PM stand for depleted and primitive present day mantle estimates, respectively (*4*). Dashed lines indicate mixing of melt derived from peridotite with ^{187}Os/^{188}Os = 0.125 and from pyroxenite with ^{187}Os/^{188}Os ratio indicated in italics (*10*).

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12. We thank I. A. Sigurdsson for help on a field trip in Iceland. This work was supported by Wolfgang Paul and Humboldt research awards to A.V.S. President of Russian Federation (grant HIII-150.2008.5), Russian Academy of Science (Department of Earth Sciences), Russian Basic Research Foundation (grants 06-05-65234 and 06-05-65227), and Deutsche Forschungsgemeinschaft (grant HO 1026/16-1) are also acknowledged for partial support to A.V.S. and V.G.B.

Supporting Online Material

www.sciencemag.org/cgi/content/full/321/5888/536/DC1
Materials and Methods
Table S1
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31 March 2008; accepted 22 May 2008
10.1126/science.1158452

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An Argonaute Transports siRNAs from the Cytoplasm to the Nucleus

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Ribonucleoprotein complexes consisting of Argonaute-like proteins and small regulatory RNAs function in a wide range of biological processes. Many of these small regulatory RNAs are predicted to act, at least in part, within the nucleus. We conducted a genetic screen to identify factors essential for RNA interference (RNAi) in nuclei of *Caenorhabditis elegans* and identified the Argonaute protein NRDE-3. In the absence of small interfering RNAs (siRNAs), NRDE-3 resides in the cytoplasm. NRDE-3 binds siRNAs generated by RNA-dependent RNA polymerases acting on messenger RNA templates in the cytoplasm and redistributes to the nucleus. Nuclear redistribution of NRDE-3 requires a functional nuclear localization signal, is required for nuclear RNAi, and results in NRDE-3 association with nuclear-localized nascent transcripts. Thus, specific Argonaute proteins can transport specific classes of small regulatory RNAs to distinct cellular compartments to regulate gene expression.

To identify factors specifically required for RNA interference (RNAi) in *Caenorhabditis elegans* nuclei, we chemically mutagenized worms and screened for mutant animals that failed to silence nuclear-localized RNAs in response to RNAi, but retained the ability to silence RNAs not localized exclusively in the nucleus (for details see fig. S1) (*1*). We identified 55 mutant alleles that were categorized into three complementation groups. Forty-six of these alleles defined a gene that we termed *nuclear RNAi defective-3* (*nrde-3*). We mapped *nrde-3* to a <1-centimorgan (cM) interval. Open reading frame (ORF) R04A9.2 lies within this genetic interval. R04A9.2 is predicted to encode an Argonaute-like protein containing a bipartite nuclear localization signal (NLS), and a PAZ and a PIWI domain. The PIWI domain of R04A9.2 lacks the DDH catalytic triad of amino acids considered necessary for Argonaute-based Slicer activity: The synonymous residues are EVQ in R04A9.2 (*2*, *3*). Sequencing of R04A9.2 from eight independent *nrde-3* alleles identified seven mutations in R04A9.2 coding sequences (Fig. 1A). Three alleles are predicted to stop translation upstream of the PAZ and PIWI domains of R04A9.2 and, thus, are likely to reveal the null phenotype. Transformation of wild-type R04A9.2 DNA into *nrde-3* mutant animals rescued phenotypes associated with *nrde-3* mutant animals (see below). Thus, R04A9.2 corresponds to *nrde-3*.

A fusion gene of green fluorescent protein (GFP) and full-length NRDE-3 under control of the endogenous *nrde-3* promoter (*1*) rescued *nrde-3* mutant phenotypes, was expressed in most somatic cells after the ~80-cell stage of development, and localized predominantly to the nucleus (Fig. 1B and fig. S2). Immunoprecipitation of NRDE-3 and isolation of coprecipitating RNAs allowed us to clone 465 RNAs of approximately 20 to 22 nucleotides that associated with NRDE-3 in vivo. These RNAs represent endogenous (endo) small interfering RNAs (siRNAs); they map to similar chromosomal loci as previously identified endo siRNAs (table S1). Endo siRNAs are thought to mediate, or to be the consequence of, ongoing negative regulation of endogenously expressed RNAs by RNAi (*4*, *5*). In *eri-1*$^{-}$, *ergo-1*$^{-}$, *mut-7*$^{-}$, *rde-4*$^{-}$, or *mut-2*$^{-}$ animals, which exhibit defects in endo siRNA production (*4*, *5*), NRDE-3 failed to associate with endo siRNAs (*1*) (Fig. 1B). In these mutant backgrounds, NRDE-3 localized to the cytoplasm, which suggests that siRNAs are necessary to localize NRDE-3 to the nucleus (Fig. 1B).

To examine this possibility, we mutated residues within the NRDE-3 PAZ domain (Y463A, Y464A) known to be necessary for siRNA-binding in related Argonaute proteins [termed NRDE-3(*PAZ)] (*6*, *7*). NRDE-3(*PAZ) failed to interact with siRNAs and localized predominantly to the cytoplasm (Fig. 1C). Exposure of animals lacking endo siRNAs (due to loss of ERI-1 activity) to exogenous double-stranded RNA (dsRNA) caused NRDE-3 to relocalize to the nucleus and induced association of NRDE-3 with exogenous (exo) siRNAs (Fig. 1D). The nuclear redistribution of NRDE-3 required NRDE-3–siRNA interactions: NRDE-3(*PAZ) failed to localize to the nucleus in response to dsRNA treatment, and NRDE-3 relocalization was dependent on RDE-4, a dsRNA binding protein necessary for converting experimentally introduced dsRNAs to exo siRNAs (*8*) (Fig. 1D). We conclude that NRDE-3–siRNA binding is necessary and sufficient for NRDE-3 nuclear redistribution.

NRDE-3 is essential for silencing nuclear-localized RNAs. A *pes-10::GFP* transgene expresses a transcript that, for unknown reasons, accumulates in the nucleus during early (<16-cell) embryogenesis. RNAi targeting the *pes-10::GFP* transcript triggered a NRDE-3–dependent loss of nuclear-localized *pes-10::GFP* RNA (*9*) (Fig. 2A and fig. S3). NRDE-3 is also required for RNAi of endogenous nuclear-localized RNAs. The *lin-15b* and *lin-15a* genes are transcribed as a bicistronic pre-mRNA that is spliced within the nucleus into distinct *lin-15b* and *lin-15a* mRNAs (*10*). Animals harboring mutations in both *lin-15b* and *lin-15a*, but not either gene alone, exhibit a multivulva (Muv) phenotype (*11*, *12*). RNAi targeting *lin-15b* results in a low percentage of ani-

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Table 1. NRDE-3 preferentially targets nuclear-localized RNAs. Animals of the indicated genotypes were fed bacteria expressing indicated dsRNAs (e.g., *unc-22*). Phenotypes (e.g., twitcher) of *eri-1(mg366)* animals exposed to control or dsRNA were defined as 0 and 4, respectively (n = 3, ± SD). NS, not scored. Asterisk indicates phenotype predicted to be elicited by silencing of nuclear-localized RNA.

	dsRNA (phenotype scored)						
Genotype	*unc-22* (twitcher)	*unc-15* (paralysis)	*pos-1* (lethality)	*unc-73* (paralysis)	*dpy-13* (dumpy)	*lir-1* (lethality*)	*lin-15b* (multivulva*)
eri-1(mg366)	3.9 ± 0.2	4	3.8 ± 0.4	3.9 ± 0.2	3.9 ± 0.1	4	4
eri-1(mg366);nrde-3(gg066)	3.7 ± 0.4	3	4	1.6 ± 0.8	1.8 ± 0.4	0	0
eri-1(mg366);rde-1(ne219)	0	0	0	0	0	0	0
eri-1(mg366);rde-4(ne301)	0	0	0	0	0	0	0
eri-1(mg366);rrf-1(pk1417)	0	NS	4	0	NS	0	0†

†n = 1.

mals exhibiting a Muv phenotype, consistent with nuclear silencing of the *lin-15* bicistron (*9*). To sensitize this assay, we repeated these experiments in a strain of *C. elegans* (*eri-1*$^{-}$) that exhibits enhanced sensitivity to dsRNAs (*13*). Of *eri-1*$^{-}$ animals exposed to dsRNA targeting *lin-15b*, 95 ± 4% exhibited a Muv phenotype, supporting the hypothesis that nuclear-localized *lin-15* bicistronic RNA can be targeted by RNAi (Table 1). Consistent with these results, RNAi targeting *lin-15b* in *eri-1*$^{-}$ animals triggered a decrease in both *lin-15b* and *lin-15a* pre-mRNA abundance (Fig. 2B). These effects were also observed in wild-type animals but were less pronounced than in *eri-1*$^{-}$ animals. NRDE-3 is required for these phenomena; in response to *lin-15b* RNAi, *eri-1*$^{-}$;*nrde-3*$^{-}$ animals did not exhibit a Muv phenotype (Table 1) and failed to silence the *lin-15b* and *lin-15a* pre-mRNAs (Fig. 2B). NRDE-3–dependent silencing of *lin-15b* pre-mRNA accounted for ~80% of total *lin-15b* silencing elicited by *lin-15b* RNAi (Fig. 2B). Finally, we, and others, have detected RNAi-triggered silencing of nuclear-localized *lir-1–lin-26* polycistronic RNA (*1*, *14*). NRDE-3 is required for this silencing (Table 1). We conclude that NRDE-3 is required for silencing of many, if not all, nuclear-localized RNAs.

Of the 465 NRDE-3–associated endo siRNAs, 33 exhibited sequence complementarity to the E01G4.5 ORF (table S1). Northern blot analysis confirmed that NRDE-3 interacts, in vivo, with E01G4.5 endo siRNAs (fig. S4). *nrde-3*$^{-}$ animals expressed E01G4.5 endo siRNAs at approximately wild-type levels (Fig. 2C). The E01G4.5 pre-mRNA and mRNA, however, were up-regulated four to five times in *nrde-3*$^{-}$ animals (Fig. 2D). *eri-1*$^{-}$ animals [which failed to express E01G4.5 siRNAs (Fig. 2C)] exhibited a similar misregulation of the E01G4.5 pre-mRNA, but a more dramatic (24 times) up-regulation of the E01G4.5 mRNA (Fig. 2D). These data suggest that NRDE-3 represents the primary, if not the sole, means for endogenous silencing of the E01G4.5 pre-mRNA, but other, NRDE-3–independent, mechanisms exist for silencing E01G4.5 mRNA. Thus, NRDE-3 is required for silencing of an endogenous, nuclear-localized RNA and likely functions downstream of endo siRNA production in these silencing events.

We investigated whether silencing in the nucleus is dependent on nuclear localization of NRDE-3. NRDE-3 harboring a three–amino acid substitution (K80A, R81A, K82A) within the predicted NRDE-3 NLS [termed NRDE-3(*NLS)] localized to the cytoplasm (Fig. 3A). Wild-type NRDE-3, but not NRDE-3(*NLS), rescued a Nrde phenotype associated with *nrde-3*$^{-}$ animals (Fig. 3B). Introduction of a heterologous simian virus 40 (SV40) NLS to NRDE-3(*NLS) restored NRDE-3^{+} function and nuclear localization to NRDE-3(*NLS) (Fig. 3, A and B). Thus, NRDE-3 contains a functional NLS, and NRDE-3 must localize to the nucleus to trigger nuclear RNAi. NRDE-3(*NLS), which localized to the cytoplasm, associated with siRNAs to an extent sim-

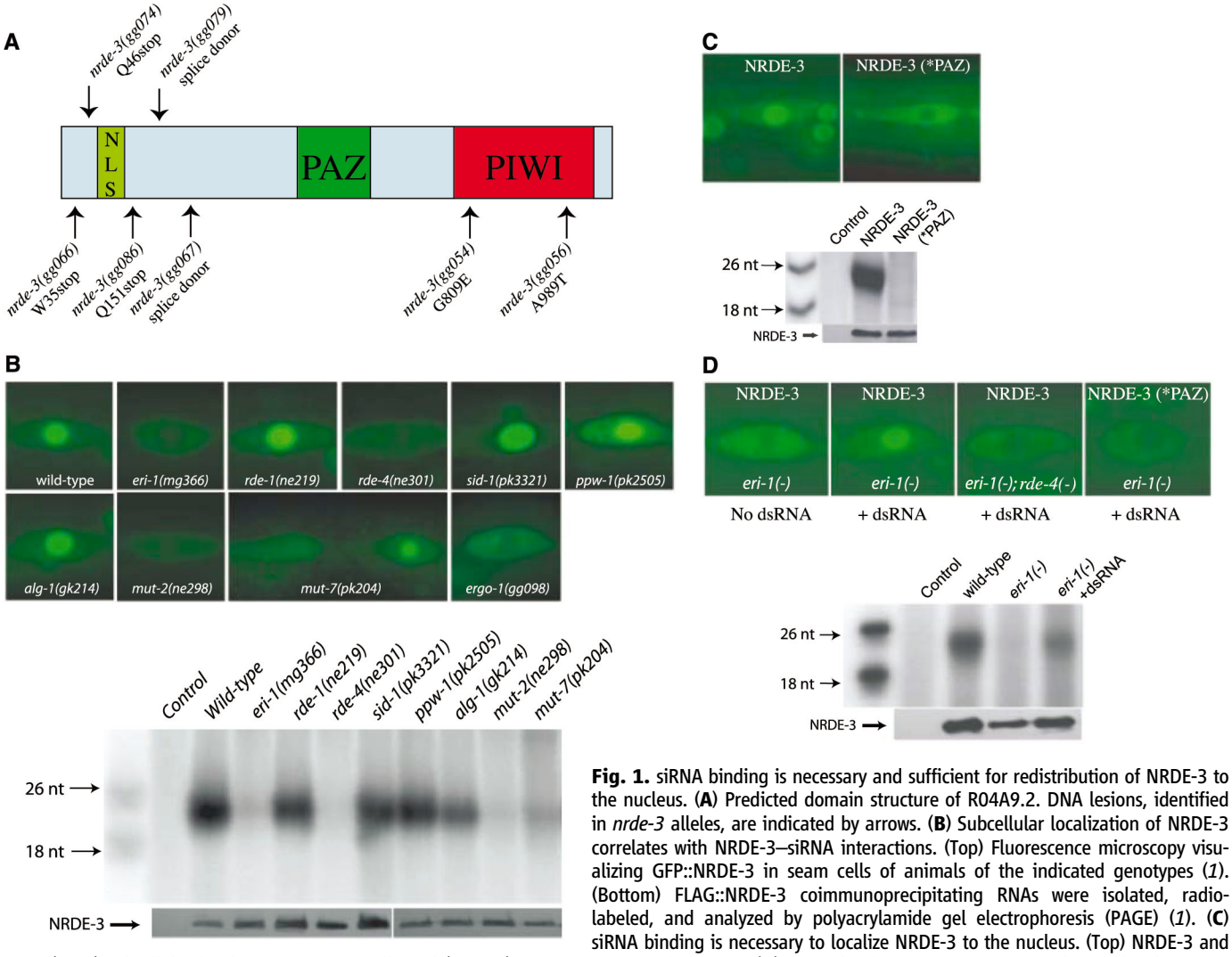

Fig. 1. siRNA binding is necessary and sufficient for redistribution of NRDE-3 to the nucleus. (**A**) Predicted domain structure of R04A9.2. DNA lesions, identified in *nrde-3* alleles, are indicated by arrows. (**B**) Subcellular localization of NRDE-3 correlates with NRDE-3–siRNA interactions. (Top) Fluorescence microscopy visualizing GFP::NRDE-3 in seam cells of animals of the indicated genotypes (*1*). (Bottom) FLAG::NRDE-3 coimmunoprecipitating RNAs were isolated, radiolabeled, and analyzed by polyacrylamide gel electrophoresis (PAGE) (*1*). (**C**) siRNA binding is necessary to localize NRDE-3 to the nucleus. (Top) NRDE-3 and NRDE-3(*PAZ) subcellular localization in seam cells and (bottom) coprecipitating endo siRNAs. (**D**) siRNA binding is sufficient for nuclear redistribution of NRDE-3. (Top) GFP::NRDE-3 localization in seam cells and (bottom) FLAG::NRDE-3 coprecipitating exo siRNAs, in animals lacking endo siRNAs, 36 hours after exposure to control bacteria or dsRNA-expressing bacteria (*1*). (-) indicates superscript minus.

ilar to that of wild-type NRDE-3, which indicated that NRDE-3 interacts with siRNAs in the cytoplasm (Fig. 3C).

Finally, RNAi targeting *lin-15b* and *unc-40* induced an association between NRDE-3 and unspliced *lin-15b* and *unc-40* RNAs, respectively (Fig. 3D and fig. S5A). NRDE-3–pre-mRNA interactions were siRNA-dependent and occurred in the nucleus: NRDE-3(*PAZ) and NRDE-

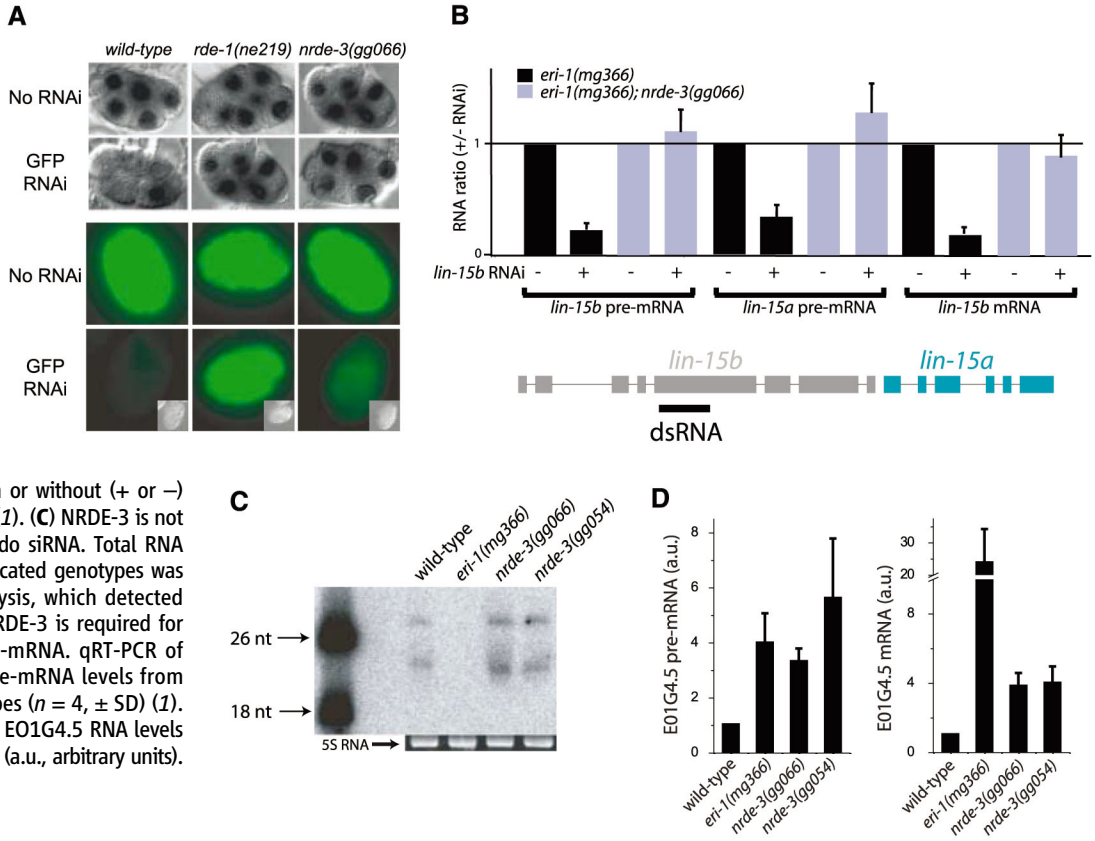

Fig. 2. NRDE-3 is required for nuclear RNAi. (**A**) NRDE-3 is essential for silencing of the nuclear-localized *pes-10::GFP* RNA. (Top) Light microscopy of ~6 cell embryos subjected to in situ hybridization targeting the *pes-10::gfp* RNA with or without GFP dsRNA (*1*). (bottom) Fluorescence microscopy of ~300 cell embryos with or without GFP dsRNA. (**B**) NRDE-3 is required for RNAi-driven silencing of nuclear-localized *lin-15* bicistronic RNA. Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) analysis of *lin-15a/b* pre-mRNA and *lin-15b* mRNA levels in animals with or without (+ or –) *lin-15b* dsRNA (n = 4 to 8, ± SD) (*1*). (**C**) NRDE-3 is not required for production of an endo siRNA. Total RNA isolated from animals of the indicated genotypes was subjected to Northern blot analysis, which detected E01G4.5 endo siRNAs (*1*). (**D**) NRDE-3 is required for silencing of an endogenous pre-mRNA. qRT-PCR of E01G4.5 mRNA and E01G4.5 pre-mRNA levels from animals of the indicated genotypes (n = 4, ± SD) (*1*). Data are expressed as the ratio of EO1G4.5 RNA levels in wild-type versus mutant animals (a.u., arbitrary units).

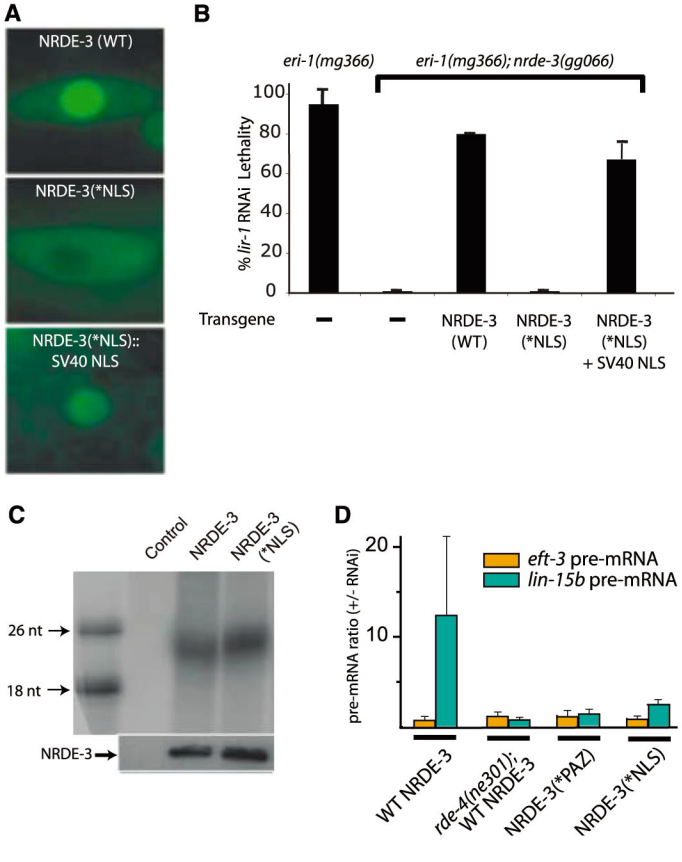

Fig. 3. NRDE-3 transports siRNAs from the cytoplasm to the nucleus: an obligatory step for nuclear RNAi. (**A**) NRDE-3 contains a functional NLS. Fluorescence microscopy of seam cells of animals expressing GFP::NRDE-3 and its variants (*1*). (**B**) Nuclear localization of NRDE-3 is required for nuclear RNAi. *lir-1* RNAi-mediated lethality results from silencing of the nuclear-localized *lir-1–lin-26* polycistronic RNA (*1*, *14*). L1 animals of the indicated genotypes expressing the indicated NRDE-3 variants were fed bacteria expressing *lir-1* dsRNA for 60 hours and scored for viability (n = 3, ± SD). (**C**) NRDE-3 associates with siRNAs in the cytoplasm. FLAG::NRDE-3– and FLAG::NRDE-3 (*NLS)–associated endo siRNAs were purified and labeled as described in Fig. 1B. (**D**) NRDE-3 associates with pre-mRNA in an RNAi-dependent manner. FLAG::NRDE-3 was immunoprecipitated from animals expressing indicated NRDE-3 variants (+/–) exposure to *lin-15b* dsRNA. cDNAs generated from associating RNAs (n = 4, ± SD) were analyzed by qRT-PCR (*1*). Data are expressed as the ratio of coprecipitating *lin-15b* RNA levels (+/–) RNAi. Similar levels of NRDE-3 were immunoprecipitated in each experiment (not shown in figure) and similar results were obtained following *unc-40* RNAi (fig. S5A).

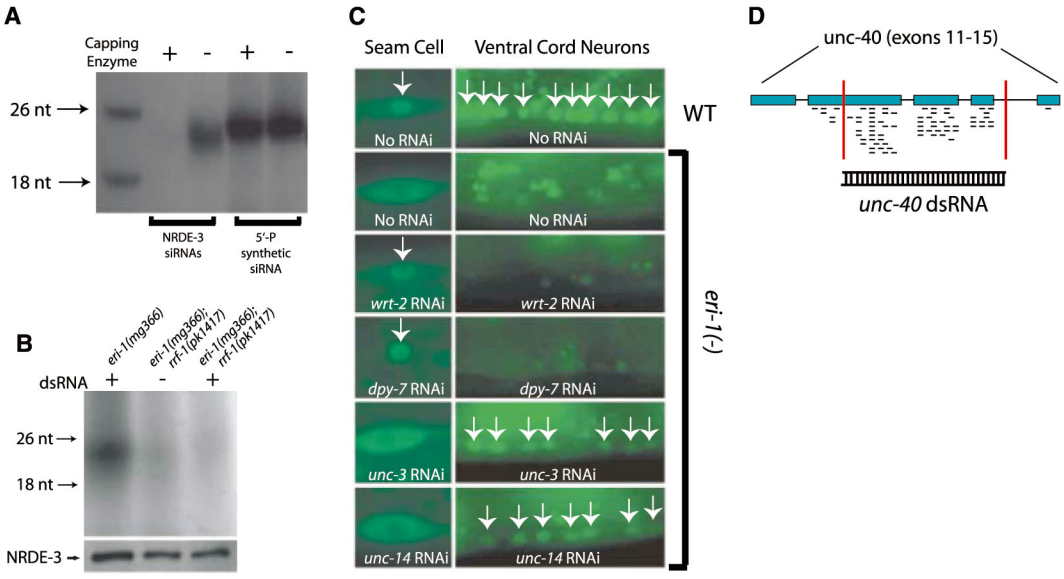

Fig. 4. NRDE-3–associated siRNAs are generated by RdRPs acting on cytoplasmic mRNA targets. (**A**) NRDE-3–associated siRNAs carry 5′ di- or triphosphates. FLAG::NRDE-3–associated endo siRNAs were treated (+) or not (–) with guanylyl transferase and GTP and then detected as described in Fig. 1B. (**B**) Animals of indicated genotypes were fed (+) or not fed (–) bacteria expressing *unc-22* dsRNA for 24 hours, and FLAG::NRDE-3–associated RNAs were detected as described in Fig. 1B. (**C**) *eri-1(mg366)* animals were fed bacteria expressing indicated dsRNAs. After 16 hours, GFP::NRDE-3 localization was assessed. Arrows indicate nuclear localization. (**D**) *eri-1(mg366)* animals were exposed to *unc-40* dsRNA (targeted sequences indicated by vertical red lines), and FLAG::NRDE-3–associated siRNAs were cloned (*1*). Short black lines represent *unc-40* sequences identified.

3(*NLS) failed to associate with pre-mRNA to an extent similar to that of wild-type NRDE-3. RNAi-driven association of NRDE-3 with pre-mRNA is consistent with a role for NRDE-3 in nuclear silencing events and may provide insights into the mechanism of nuclear silencing in *C. elegans*. Taken together, these data indicate that NRDE-3 can escort endo and exo siRNAs from the cytoplasm to the nucleus in an NLS-dependent manner, and that this translocation step is essential for silencing nuclear-localized RNAs.

NRDE-3 is required for silencing E01G4.5 pre-mRNA, but only partially required for endogenous RNAi-mediated silencing of E01G4.5 mRNA, which suggests that NRDE-3 may not be required for cytoplasmic silencing events (Fig. 2D). Three lines of evidence support this hypothesis. First, expression of NRDE-3(*NLS) did not enhance RNAi responses: *nrde-3*$^{-}$, as well as *nrde-3*$^{-}$ animals expressing NRDE-3(*NLS), responded similarly to RNAi (fig. S5, B and C). Second, *nrde-3*$^{-}$ animals retained the ability to respond (albeit at somewhat attenuated levels) to RNAi targeting RNAs (such as *pos-1*, *unc-22*, *unc-15*, *unc-73*, and *dpy-13*) not localized exclusively to the nucleus (Table 1). Incidentally, the near–wild-type response of *nrde-3*$^{-}$ animals to these dsRNAs indicates that (for unknown reasons) NRDE-3–dependent silencing is not always a significant component of RNAi responses after typical feeding RNAi experiments in *C. elegans*. Finally, at the >16-cell stage of embryonic development, the *pes-10::GFP* RNA is transported to the cytoplasm (*9*). Late stage (~300-cell) *nrde-3*$^{-}$ embryos, exposed to GFP dsRNA, retained the ability to partially silence PES-10::GFP (Fig. 2A, bottom). Thus, whereas NRDE-3 is required for RNAi of nuclear-localized RNAs, it is not required for all RNAi-related silencing events and likely does not play a significant role in silencing of cytoplasmic mRNAs.

We next examined NRDE-3–associated siRNAs in more detail. About 85% of NRDE-3–associated siRNAs are substrates for in vitro RNA capping reactions catalyzed by guanylyl transferase, indicating that NRDE-3–associated siRNAs carry 5′ di- or triphosphates (Fig. 4A). RNA-dependent RNA polymerases (RdRPs) are thought to amplify RNAi signals by utilizing RNA molecules, which have been targeted by RNAi, as templates for transcription of additional siRNAs. The presence of 5′ di- or triphosphates on NRDE-3–associated siRNAs suggested that these siRNAs might be generated by RdRP action (*15*, *16*). Three lines of evidence support this hypothesis. First, the RdRP, RRF-1 was required for silencing of the *lir-1–lin-26* polycistronic RNA in response to *lir-1* RNAi (Table 1). In addition, in *rrf-1*$^{-}$ animals NRDE-3 failed to associate with exo siRNAs after RNAi (Fig. 4B). Second, acute exposure (16 hours) of animals lacking endo siRNAs to dsRNAs targeting *wrt-2* and *dpy-7*, two genes expressed in hypodermal cells, triggered a nuclear redistribution of NRDE-3 in hypodermal cells, but failed to initiate NRDE-3 translocation in nonhypodermal cells (Fig. 4C). RNAi targeting *unc-14* and *unc-3*, two genes expressed in ventral cord neurons, triggered NRDE-3 redistribution predominantly in ventral cord neurons (Fig. 4C). Thus, nuclear redistribution of NRDE-3 appears to require expression of RNA molecules exhibiting sequence similarity to the trigger dsRNA (*1*). Third, exposure of animals lacking endo siRNAs to dsRNA targeting the *unc-40* gene induced association of NRDE-3 with siRNAs that are exclusively antisense to the *unc-40* mRNA. Of these siRNAs, 10% were derived from *unc-40* sequences not covered by the *unc-40* dsRNA to which the animals were exposed (Fig. 4D). NRDE-3 associated with *unc-40* siRNAs that exhibited complementarity exclusively to *unc-40* exonic sequences despite the fact that both exonic (82%) and intronic (18%) *unc-40* sequences were targeted by RNAi in these experiments (Fig. 4D). A similar bias toward exonic sequences was observed in NRDE-3–associated endo siRNAs (table S1). These data, in conjunction with our data demonstrating that NRDE-3 associates with siRNAs in the cytoplasm, strongly suggest that NRDE-3 associates predominantly with siRNAs generated by RdRPs acting on mRNA templates in the cytoplasm and that this association is essential for nuclear RNAi.

Our data show that nuclear silencing events in *C. elegans* are the result of transport of siRNAs, and not long dsRNAs, to the nucleus via an active transport system involving Argonaute proteins. *nrde-3*$^{-}$ animals are defective for all nuclear silencing phenomena that we have assayed, which indicates that other mechanisms of siRNA nuclear localization, such as passive diffusion, and/or nuclear envelope breakdown during mitosis are unlikely to be major contributors to these processes. Many small regulatory RNAs, such as scanRNAs in ciliated protozoa, heterochromatin-related siRNAs in plants and fungi, and piRNAs in mammals, are thought to function, at least in part, within the nucleus (*17*–*20*). It will be interesting to determine whether these small regulatory RNAs are transported to the nucleus by mechanisms similar to the one described here.

References and Notes

1. Materials and methods are available as supporting material on *Science* Online.
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Supporting Online Material
www.sciencemag.org/cgi/content/full/321/5888/537/DC1
Materials and Methods
Figs. S1 to S5
Table S1
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11 March 2008; accepted 28 May 2008
10.1126/science.1157647

Entangling the Spatial Properties of Laser Beams

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Position and momentum were the first pair of conjugate observables explicitly used to illustrate the intricacy of quantum mechanics. We have extended position and momentum entanglement to bright optical beams. Applications in optical metrology and interferometry require the continuous measurement of laser beams, with the accuracy fundamentally limited by the uncertainty principle. Techniques based on spatial entanglement of the beams could overcome this limit, and high-quality entanglement is required. We report a value of 0.51 for inseparability and 0.62 for the Einstein-Podolsky-Rosen criterion, both normalized to a classical limit of 1. These results are a conclusive optical demonstration of macroscopic position and momentum quantum entanglement and also confirm that the resources for spatial multimode protocols are available.

Position and momentum are a fundamental example of conjugate quantum observables. Optical measurements of this conjugate pair are ubiquitous throughout many fields of research and across a broad range of scales. Applications in optics span from biology to astronomy, from the nanometer regime with atomic force microscopy (*1*) and optical tweezing (*2*) to the kilometer regime with free-space optical communication (*3*) and interferometry (*4*, *5*). These applications require continuous sampling of the data, although their accuracy is fundamentally limited by the quantum noise of these beams. Special squeezed beams, with the noise suppressed in one quadrature, have been used to improve the properties of many optical instruments (*6*), improving the signal-to-noise ratio for one selected observable.

Quantum entanglement, where two systems are quantum-correlated, can allow a near-perfect prediction from one system to the other and can enable new techniques for using information from one system to act on the other, thereby overcoming the limits set by quantum noise. In optics the systems are beams of light, with a single mode of electromagnetic radiation describing all their properties. Each beam can be represented by a single quantum operator. Such single-mode continuous wave (CW) optical beams have already been used to demonstrate strong entanglement between the amplitude and phase quadrature of pairs of beams (*7*–*9*) or the polarization of the beams (*10*–*13*). In combination with feed-forward control, these beams can be used for applications such as entanglement distillation (*14*) and teleportation (*15*).

However, laser beams have additional spatial properties that are described by higher-order spatial modes. A multimode description contains more information and allows the coding of more complex, multidimensional quantum information (*16*). The experimental techniques are direct and reliable because of the link between spatial properties and the well-known basis of Hermite-Gaussian transversely excited laser modes (TEM_{ij}, where i and j are the order numbers in the x and y directions). For a CW beam with the energy in a pure TEM_{00} mode (the reference beam) and detectable power of microwatts to milliwatts, the simplest spatial modulation is a displacement of the entire mode in the transverse directions x and y. For a periodic displacement at a frequency Ω, where the size of the displacement is much smaller than the diameter ω_0 of the beam, all of the information about the displacement $X(\Omega)$ is contained in the real part (or amplitude quadrature) of the mode TEM_{10}, whereas the orthogonal displacement $Y(\Omega)$ appears in the real part of TEM_{01}. The accuracy of the measurement of the position of the beam is limited by quantum noise in the modes TEM_{10} and TEM_{01}. Previously, we have generated squeezed light in these higher-order modes (*17*). This technique of synthesizing is different from the idea of entangling images and complements the generation of intensity using four-wave mixing in an atomic vapor where a large number of modes can be entangled (*18*).

Concentrating on one transverse direction, the displacement X of the beam forms a conjugate pair of observables with the direction θ of the beam. The information for the direction is contained in the imaginary part (or phase quadrature) of the corresponding higher-order mode. This can be seen by the following expression for the electric field distribution $E(X, \theta)$ of a TEM_{00} mode that is both displaced by X and tilted by θ with the spatial information in the TEM_{10} mode (*19*)

$$E(X,\theta) \approx \mathrm{TEM}_{00} + \frac{X}{\omega_0}\mathrm{TEM}_{10} + i\frac{\omega_0\pi\theta}{\lambda}\mathrm{TEM}_{10} \quad (1)$$

where ω_0 is the waist diameter, i is the imaginary unit, and λ is the wavelength of the beam. The direction of the laser beam θ corresponds to the transverse momentum of the photons in the beam. An improvement in the spatial accuracy beyond the quantum noise limit (QNL) for either position or direction measurement of a TEM_{00} beam has already been demonstrated (*6*).

We then investigated the spatial entanglement of a pair of beams (*20*). The existence of entanglement can be determined by measuring correlations between the displacements and directions of the two beams, respectively, to a level below the QNL. For beams that are coherent states, the spatial fluctuations of the two beams A and B are independent, and there are no correlations or anticorrelations. Thus, variance measurements for the sum $V(X_A + X_B)$ and difference $V(X_A - X_B)$ of the positions of the beams are

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both at the QNL. Similarly, the sum $V(\theta_A + \theta_B)$ and difference $V(\theta_A - \theta_B)$ of the directions of the beams are at the QNL. This is illustrated in Fig. 1, where the outer box shows the QNL for such independent beams. Inside the QNL box in this figure, the results are also shown for the entangled case. As well as measuring correlations between the spatial properties of the beams directly, we can infer the properties of B from a measurement of A, or vice versa, with an improved accuracy below the QNL. A measurement of these inferences allows us to demonstrate the Gedanken experiment discussed by Schrödinger and Einstein *et al.* (*21*), who proposed the link between the observables in two systems, and to quantify the extent of the entanglement.

The entangled beams are created by combining a spatially squeezed reference beam (TEM_{00} copropagating with squeezed TEM_{10}) with another squeezed beam (TEM_{10}) on a 50:50 beamsplitter. If both of the entangled beams were in a single spatial mode and the relative phase of the beams was locked to $\phi_c = \pi/2$, we would have a conventional entangler (*22*). However, in our case the spatial information of the strong reference beam is now distributed into both output beams A and B, together with the quantum entanglement created by the two squeezed higher-order modes. Thus, the quantum entanglement can be measured by using two balanced homodyne detectors (HDs), each with carefully mode matched TEM_{10} modes (Fig. 2). By selecting the phases of the two local oscillator beams (ϕ_{LOA} and ϕ_{LOB}), we can choose to measure either the differential position or differential momentum of the two beams. We used optical parametric amplifiers (OPAs) as the generators of the squeezed light. In this experiment, the OPAs are linear resonators—each with one $LiNbO_3$ nonlinear crystal—and operate with a pump wavelength of 532 nm and a seed of 1064 nm. The entire experiment is driven by one low-noise monolithic Nd-YAG (Nd–yttrium-aluminum-garnet) laser with a second harmonic generator, with all beams phase-locked to each other. The OPAs generate up to –3.8 dB of squeezing when tuned to TEM_{10} (–5 dB for TEM_{00}), and the technical noise level from the custom made photodetectors is –14 dB, compared to the QNL. To avoid technical noise from the laser at low frequencies and the various modulation frequencies that we need for the locking loops that maintain the correct beam conditions, we concentrated on measurements at Ω = 3 to 4 MHz.

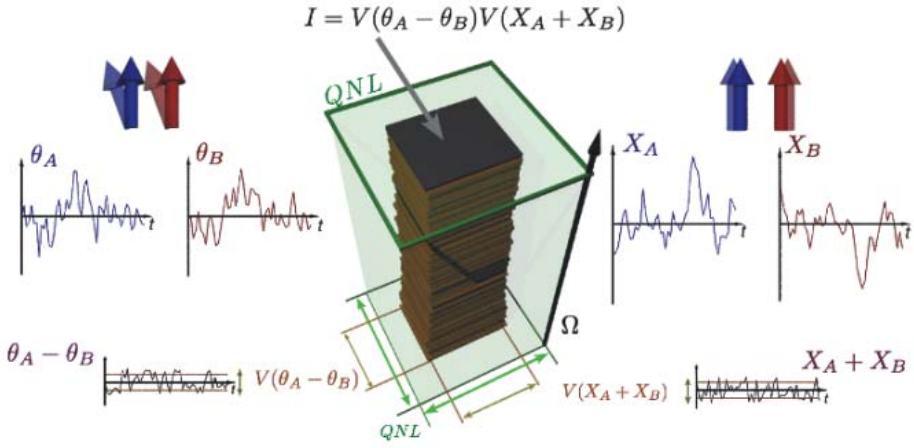

Fig. 1. No laser beam can have a fixed position or momentum. Spatial entanglement manifests itself as a strong quantum correlation between the position and direction of two beams, A (blue) and B (red). On the left, this illustration shows the fluctuating directions θ_A and θ_B of the two beams, which are correlated, and on the right, it shows the positions X_A and X_B, which are anticorrelated. For perfectly entangled beams, the differences $(\theta_A - \theta_B)$ and $(X_A + X_B)$ would both be zero. Real entangled beams have a small residual differential movement. The variances $V(X_A + X_B)$ and $V(\theta_A - \theta_B)$ are calibrated against their respective QNL, which corresponds to the differential movement of two laser beams with independent quantum noise. A good measure of entanglement is the inseparability, which (for a symmetric system) is the product $I = V(X_A + X_B)V(\theta_A - \theta_B)$. This is shown as the area of the filled rectangles in the center of this figure. Each slice of the tower represents one measurement, and the comparison of the area with the QNL (green box) directly shows the degree of inseparability.

Entanglement can be quantified in a variety of ways, each motivated by a specific physical property of the entangled beams. For entangled beams produced from beams with infinite squeezing and measured with perfect efficiency, all of these measures give a value of zero. For the practical case of imperfect entanglement due to the finite degree of squeezing available and the limited detection efficiency, the different measures give different values. A measure that is simple to implement is the degree of inseparability between the two beams. This can be quantified by the product of the normalized variances of the sum of

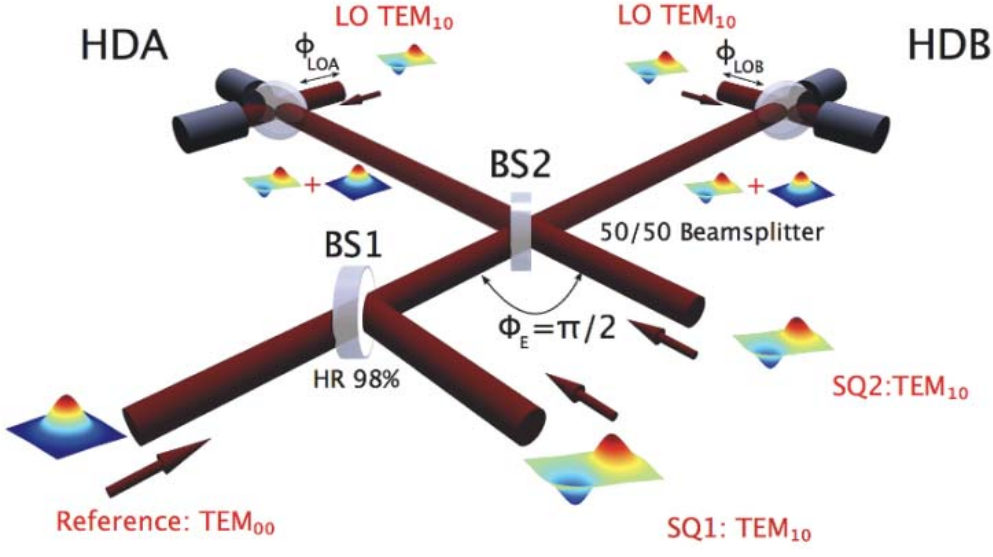

Fig. 2. Schematic for our experiment. A reference TEM_{00} beam is first combined on beamsplitter BS1 (high reflectivity 98%) with a squeezed TEM_{01} mode (SQ1), producing a spatially squeezed beam. This is entangled with a second squeezed TEM_{01} mode on beamsplitter BS2 (reflectivity 50%). The two entangled beams are detected separately with two homodyne detectors (HDA and HDB) using local oscillator (LO) beams in the TEM_{01} mode.

the beam positions, $V(X_A + X_B)$, and the difference of the beam directions, $V(\theta_A - \theta_B)$. For a simple symmetric arrangement, the condition for inseparability is

$$I = V(X_A + X_B)\cdot V(\theta_A - \theta_B) < 1 \quad (2)$$

The central tower of Fig. 1 shows the actual experimental spectra for the two variances resulting in $I = V(X_A + X_B)V(\theta_A - \theta_B)$ for a scan from 3 to 4 MHz. It is directly apparent from Fig. 1 that both variances are below the QNL, so the directions are correlated and the positions are anticorrelated below the QNL. The two beams are spatially entangled, and we obtain a result of $I = 0.51 \pm 0.02$ at 3.3 MHz. Not only is the product of the variances <1, but we also find that $V(X_A + X_B) = 0.82 \pm 0.03$ and $V(\theta_A - \theta_B) = 0.76 \pm 0.01$ independently, which is the requirement for a lowest-order cluster state of only two neighboring elements (*23*).

A more complex measure of entanglement is the evaluation of the Einstein-Podolsky-Rosen (EPR) criterion (*24*). This can be measured directly by evaluating the conditional variance of the measurements of X_A and θ_A from one beam and X_B and θ_B from the other beam, as was measured with the use of single photons in (*25*). The condition for entanglement is given by the conditional variance

$$\varepsilon = V(X_B \mid X_A)V(\theta_B \mid \theta_A) < 1 \quad (3)$$

The direct detection of the conditional variance is not possible with an electronic spectrum analyzer, and the value of ε can be calculated with several different combinations of variances from measurements of the two beams (*20*). More details of this process are provided in the supporting online material (SOM). Figure 3 shows ε(Ω) plotted for a range of frequencies. We find strong EPR entanglement for a broad frequency interval, with a value of $\varepsilon = 0.62 \pm 0.03$ at 3.3 MHz.

Practical systems suffer from asymmetries in the apparatus, where the losses on the two entangled beams are different, and in this case there are two different values for ε. For our experiment, the two values are given by $V(X_B|X_A)V(\theta_B|\theta_A)$ and $V(X_A|X_B)V(\theta_A|\theta_B)$. In the first case, we take a measurement of beam A, which has the fewest losses, and infer what we will measure on beam B. This is the result that has been quoted above, and more details are given in the SOM. The other case, where we take beam B with its higher losses, and attempt to infer beam A, gives us a value for ε of 0.94. The two different cases, with experimental data, are shown in Fig. 3. Finally, we must test the quality of the intensity mode of the entangled beams before we can claim to have spatial entanglement for our laser beams. The beam intensity must be dominantly in the TEM_{00} mode, as this is the mode for which we are measuring the position and momentum. Our entangled beam is close to being entirely TEM_{00} mode, with over 90% of the intensity being in the TEM_{00} mode.

We have entangled the position and momentum of laser beams by combining squeezed TEM_{10} with the original beam. By combining other higher-order squeezed modes TEM_{ij} with the reference beam TEM_{00}, we can entangle beams that carry other spatial information with reduced quantum fluctuations. Additionally, by using homodyne detection with a local oscillator in the mode TEM_{ij}, we can detect one specific spatial property of the reference TEM_{00} beams (*16*), or each mode independently. We have achieved a value for the EPR parameter that is a genuine proof of the entanglement of position and momentum of two laser beams.

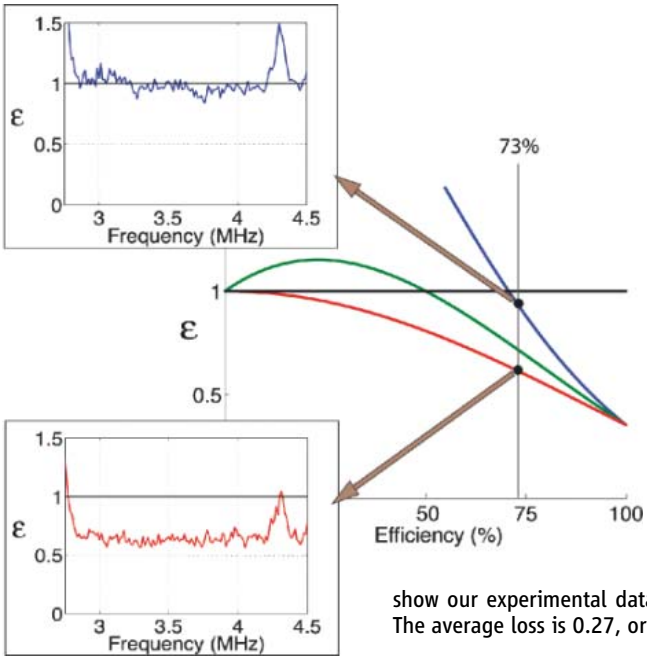

Fig. 3. The effect of asymmetry. Theoretical curves and measured EPR traces for beam position and direction in a system with different losses on the way to and in the two HDs. The central image shows a theoretical curve for the degree of EPR entanglement for varying total loss. The three cases are (i) upper curve, we measure the beam that encounters fewer losses and infer the other beam; (ii) middle curve, a symmetric system where we measure either beam and infer the other; and (iii) lower curve, we measure the beam with lower losses and infer the other beam. The two insets at left show our experimental data for the two practical cases. The average loss is 0.27, or an efficiency of 0.73.

This technology can be used as a resource for new quantum information applications, particularly those that require multimode entanglement. With this system of multiple modes we can create complex correlations within one beam. For example, we can start with several squeezed sources for TEM_{ij} and consider the creation of the equivalent of cluster states by correlating these modes using spatial techniques, in analogy to the creation of such states from several TEM_{00} beams (*26*). The spatial techniques may provide advantages in the simplicity of such systems. In addition, these complex quantum correlations can be sent in free-space communication in one beam or can potentially be stored as multimode spatial information (*27*).

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28. This work was funded by the Australian Research Council Centre of Excellence Scheme.

Supporting Online Material

www.sciencemag.org/cgi/content/full/321/5888/541/DC1
SOM Text
Fig. S1

25 April 2008; accepted 26 June 2008
10.1126/science.1159663

Entangled Images from Four-Wave Mixing

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Two beams of light can be quantum mechanically entangled through correlations of their phase and intensity fluctuations. For a pair of spatially extended image-carrying light fields, the concept of entanglement can be applied not only to the entire images but also to their smaller details. We used a spatially multimode amplifier based on four-wave mixing in a hot vapor to produce twin images that exhibit localized entanglement. The images can be bright fields that display position-dependent quantum noise reduction in their intensity difference or vacuum twin beams that are strongly entangled when projected onto a large range of different spatial modes. The high degree of spatial entanglement demonstrates that the system is an ideal source for parallel continuous-variable quantum information protocols.

Images have always been a communication medium of choice because of the large amount of information they can carry in their details. Up to the present, optical devices have mostly ignored the phase and intensity fluctuations that result from the quantum nature of the light that transports the images. The ability of modern detectors to see the quantum fluctuations of light has triggered the emergence of the field of quantum imaging, where "visual" information can be contained not only in the local intensity of a light field but also in the local quantum fluctuations (*1*). Controlling these local fluctuations can improve optical resolution (*2*), image amplification (*3*), and beam positioning (*4*). Seen in the context of quantum information science, quantum imaging is an extension of quantum optics to the transverse spatial degrees of freedom, and it allows us to benefit from the intrinsic parallelism of image processing. Here, we demonstrate the production of highly multimode quantum-correlated "twin beams" by nondegenerate four-wave mixing (4WM) in a hot atomic vapor. The beams display continuous-variable (CV) entanglement in that the quantum fluctuations of a given pair of spatial modes cannot be described independently, a property also known as inseparability. This property is needed for the implementation of numerous quantum information protocols (*5*).

CV-entangled light can be generated with a phase-insensitive optical amplifier (*6*), which mixes and amplifies two field modes a and b (the probe and the conjugate), generating twin beams. When fed with coherent states, vacuum states, or both, the amplifier produces a pure state whose entanglement manifests itself through strong correlations between the fluctuations of the output probe and conjugate fields. These fields are each described quantum mechanically by a pair of conjugate quadrature operators: $(\hat{X}_a, \hat{Y}_a)$ for the probe and $(\hat{X}_b, \hat{Y}_b)$ for the conjugate. The variance of these operators for coherent states sets the quantum noise level (QNL) of the quadrature fluctuations. They are normalized here so that the QNL is unity. To describe the correlations between the twin beams, we define the joint quadrature operators $\hat{X}_- = (\hat{X}_a - \hat{X}_b)/\sqrt{2}$ and $\hat{Y}_+ = (\hat{Y}_a + \hat{Y}_b)/\sqrt{2}$, which combine the quadrature operators of the individual fields. For an amplifier gain (G) larger than one, the fluctuations $\langle \Delta \hat{X}_-^2 \rangle = \langle \Delta \hat{Y}_+^2 \rangle$ are squeezed: They are smaller than the QNL. The squeezing is larger for a larger amplifier gain. Reciprocally, the observation of noise reduction with respect to the QNL on both joint quadratures $\hat{X}_-$ and $\hat{Y}_+$ for two optical fields is sufficient to prove their entanglement, or inseparability. More generally, the degree of inseparability can by quantified by $I = \langle \Delta \hat{X}_-^2 \rangle + \langle \Delta \hat{Y}_+^2 \rangle$. The states are inseparable when $I < 2$ (*7*, *8*). Inseparability has been demonstrated for single–spatial-mode twin beams, which can be likened to single pixel images, with optical parametric oscillators (OPOs) operating below (*9*, *10*) and above threshold (*11*, *12*), with 4WM in optical fibers (*13*) and with mixing of single-mode squeezed states, giving the best measured inseparability to date of $I = 0.56$ (*14*).

A multi–spatial-mode optical amplifier couples many pairs of spatial modes $\{(a_i, b_i)\}$, so that the quantum fluctuations of the twin beams depend not only on the frequency but also on the spatial variables. Multimode operation occurs naturally in amplifiers using nonlinear processes such as parametric down-conversion (PDC) [$\chi^{(2)}$ nonlinearity] or 4WM [$\chi^{(3)}$ nonlinearity], as long as the phase-matching condition allows a range of probe and conjugate wave vectors to be coupled (*15*). Multi–spatial-mode entanglement has been observed in PDC (*16*, *17*), where the gain is inherently low, via photon-counting and coincidence techniques. This can be done in spite of the weakness of the quadrature entanglement and the low detector efficiency. Here, we produce states with large quadrature entanglement that can be detected efficiently.

Large gains can be obtained with a $\chi^{(2)}$ medium in an OPO configuration, where a build-up cavity enhances the nonlinearity (*18*), but these devices have proved challenging to operate in the multi–spatial-mode regime. A possible remedy is direct synthesis of multimode quantum light (*4*, *19*). Another route, which we present here, is 4WM in atomic vapors, which display very large nonlinearities. The recent demonstration of a large quantum noise reduction in the intensity difference of twin beams generated by nondegenerate 4WM in a hot vapor of ^{85}Rb without the use of a cavity (*20*) has prompted our interest in observing spatial quantum effects in $\chi^{(3)}$ media. The scheme (Fig. 1A) follows a double-lambda configuration (*21*, *22*) in which a strong pump field of frequency ω_0 is mixed (by way of the interaction with four atomic levels) with two weak fields of frequencies ω_a and ω_b (the probe and the conjugate) such that $\omega_a + \omega_b = 2\omega_0$ (*23*). The fundamental feature of the scheme

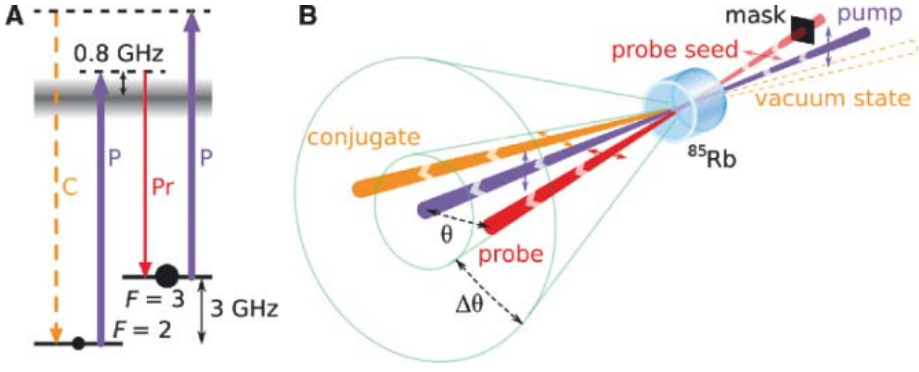

Fig. 1. 4WM in a hot ^{85}Rb vapor. (**A**) Atomic levels of the $D1$ transition of ^{85}Rb involved in the double-lambda scheme. The width of the excited state represents the Doppler broadening. The pump (P) puts most of the atomic population in the $F = 3$ hyperfine electronic ground state. The parametric process converts two pump photons into one probe (Pr) photon and one conjugate (C) photon, which leads to quantum correlations between the fields. (**B**) Geometry of the experiment. The bright pump is focused in a hot ^{85}Rb vapor cell and couples with probe and conjugate fields located symmetrically at a small angle θ with respect to the pump axis. The coupling is substantial over a range of angles $\Delta\theta$ and has a cylindrical symmetry. The coupling region is schematically represented by the green solid angle. When the process is seeded in a particular probe mode, as represented in this figure, this mode and the corresponding conjugate mode (initially in a vacuum state) emerge as bright twin beams. Arbitrarily shaped twin beams can be obtained by inserting an amplitude mask on the probe seed. The polarization of the fields is indicated by the double arrows.

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is that the light fields are coupled via the coherence between the two hyperfine ground states, with a minimum amount of atomic population in the excited state. This strongly suppresses the spontaneous emission and, along with the large detunings, reduces the absorption of the probe and the conjugate fields. In this condition, and in the limit of low pump depletion, the system behaves like a near-perfect phase-insensitive amplifier for the probe and the conjugate (*24*).

The phase-matching condition dictates that the beams in the 4WM be nearly colinear, with the probe and the conjugate propagating at the same small angle θ with respect to the pump, on either side of it (Fig. 1B). The finite length of the medium slightly relaxes the longitudinal phase-matching condition and allows for a range of angles Δθ, effectively setting the angular acceptance of the process. The angular resolution of the amplifier is the angular size of the smallest optical mode (in the far field) that is coupled to the gain medium. It is diffraction-limited by the transverse size of the gain region, which is controlled by the waist and the intensity of the pump in the vapor. From the angular acceptance and angular resolution, one can estimate that 100 orthogonal pairs of spatial modes are independently coupled in the medium (*23*).

Fig. 2. Intensity-difference squeezing in the multi–spatial-mode regime. An opaque amplitude mask is placed in the far field of the seed, and the high spatial frequencies are filtered out with a pinhole. (**A**) Image of the probe seed in the far field. (**B**) Image of the output probe in the intermediate field. (**C**) Image of the conjugate in the far field. The intensities are normalized to 1. The different optical conjugations for the probe and the conjugate images show the lensing effect due to the cross-Kerr interaction with the pump beam, which acts mostly on the probe. These images demonstrate the optical quality, in the classical sense, of the amplifier. Moreover, intensity-difference measurements on the output probe and the conjugate show that the letters N and T are independently squeezed, which proves the multimode operation of the 4WM.

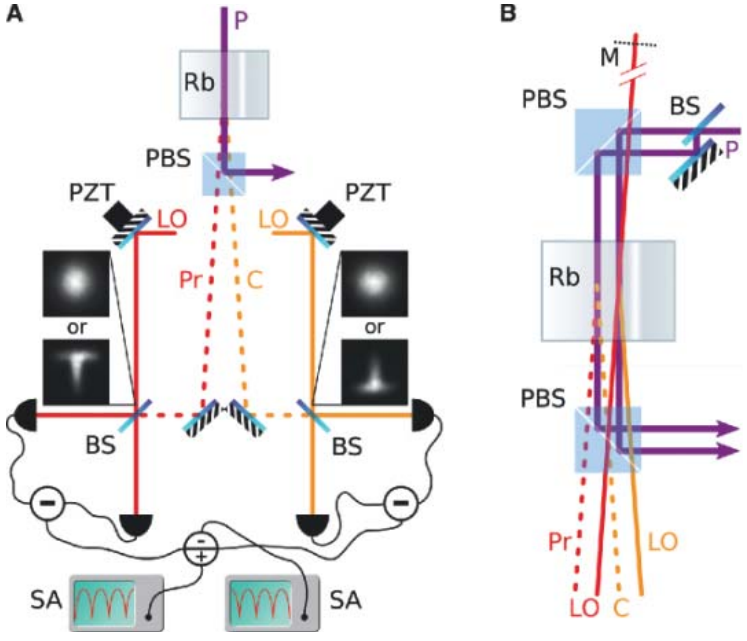

Fig. 3. Entanglement measurement with dual-homodyne detection. The two-mode squeezed vacuum, represented by dashed lines, is nondegenerate and requires two phase-locked LOs with frequencies separated by 6 GHz. (**A**) General layout. P, pump; Pr, probe; C, conjugate; BS, 50/50 beamsplitter; PBS, polarizing beamsplitter; PZT, piezoelectric actuator; Rb, Rubidium vapor cell; SA, spectrum analyzer. A hybrid junction forms the sum and difference of the quadrature signals measured by the HDs, and both the sum and difference noises are recorded simultaneously by two spectrum analyzers. The piezoelectric actuators are scanned synchronously so that the HDs measure the same quadratures at any given time. The images show two possible beam profiles for the LOs. (**B**) Detail of the generation of the LOs. The pump beam is split into two parts of equal power so that the spatial modes of the LOs, created by seeded 4WM, match a pair of entangled spatial modes created by similar unseeded 4WM. In the experiment, the probe, conjugate, and LO beams are not in the same plane, which makes their physical separation easier. An optional amplitude mask (M) placed in the far field of the seed allows for the generation of LOs of arbitrary mode shape.

One way of observing the quantum correlations is to seed the process with a coherent state of intensity I_0 at the probe frequency, whereas the input conjugate is left in a vacuum state (Fig. 1B). The amplification produces bright twin beams of intensities $I_a = GI_0$ for the probe and $I_b = (G-1)I_0$ for the conjugate. The intensity difference $I_a - I_b$ displays a theoretical normalized noise power equal to $1/(2G-1)$, below the QNL of unity. Experimentally, a gain of up to 10 and a quantum noise reduction of more than 8 dB have been observed for Gaussian twin beams (*24*). The multi–spatial-mode operation of the amplifier means that one can produce bright twin beams in any set of spatial modes fitting into the spatial bandwidth of the process and that matching subparts of the twin beams that are contained in the spatial bandwidth should be independently entangled and, in particular, should be intensity-difference squeezed (*25*).

To illustrate this point, we seeded the process with a "NT"-shaped spatial mode created with an opaque amplitude mask, and we recorded the intensity-difference noise power at 3.5 MHz with a balanced detector and a radiofrequency spectrum analyzer. The highest spatial frequencies of the seed, which would not be amplified by the 4WM, were removed with a spatial filter placed before injection in the amplifier. The overall detection efficiency is 90 ± 3% (*26*), and we corrected the measured noise for only the electronic noise floor of the photodetector. Figure 2 shows the input probe, output probe, and output conjugate in the image planes of the mask. Cross-Kerr modulation from the intense pump causes the probe beam to experience a lensing effect as it crosses the pump beam. The same effect is negligible for the conjugate because of the large detuning from the atomic transition. As a result, the image plane of the input mask is located at a different distance for the probe and the conjugate. Apertures select which part of each output beam hits the balanced detector. At a gain $G \approx 4.5$, up to –5.4 ± 0.2 dB of intensity-difference squeezing is recorded for the full NT modes, –5.2 ± 0.2 dB for the "N" subparts, and –5.1 ± 0.2 dB for the "T" subparts, showing that matching subparts of the twin beams are correlated. The smallest size of correlated subparts is determined by the angular resolution, and the intensity difference of unmatched subparts (for instance, the N of one beam and the T of the other beam) displays amplifier excess noise (*25*). The independence of the squeezing of matched local subparts is the hallmark of spatial squeezing (*27*, *28*).

Intensity measurements on bright beams give access to the fluctuations of the amplitude quadratures, which then coincide with the $\hat{X}_a$ and $\hat{X}_b$ quadratures. Alone, they cannot prove entanglement. In fact, one would also expect the phase sum of the fields to be squeezed (*6*, *28*), where the phase quadratures coincide with $\hat{Y}_a$ and $\hat{Y}_b$. Measuring all of the four quadratures $\hat{X}_a$, $\hat{Y}_a$, $\hat{X}_b$, and $\hat{Y}_b$ requires homodyning with local oscilla-

tors (LOs) mode-matched to the pair of modes under interrogation. We tuned the amplifier to have a gain $G \approx 4$, and we fed it with vacuum inputs, so as to generate a two-mode squeezed vacuum output, or vacuum twin beams (dashed lines in Fig. 3A). Two balanced homodyne detectors (HDs) whose phases are scanned synchronously measure the same quadrature on both twin beams at any given time, and the noise of the sum and the difference of the two signals is analyzed at a frequency of 0.5 MHz. The LOs must be phase-locked and separated in frequency by 6 GHz, one at the probe and the other at the conjugate frequency. As the phases are scanned, the HDs successively measure the $\hat{X}$, $\hat{Y}$, $-\hat{X}$, and $-\hat{Y}$ quadratures of both beams for every $\pi/2$ phase shift, and the difference and sum signals alternately measure the $\hat{X}_-$, $\hat{Y}_+$, $-\hat{X}_-$, and $-\hat{Y}_+$ joint quadratures. As shown in Fig. 4A, where Gaussian-shaped LOs were used, the joint quadratures are measured to be squeezed by -4.3 ± 0.2 dB, which fulfills the inseparability criterion $I = 0.74 \pm 0.02 < 2$. Taking into account the finite detection efficiency of 90%, the inferred squeezing would be –5.2 dB, and the inseparability would become $I = 0.60$. There exists a degree of entanglement higher than inseparability—called Einstein-Podolsky-Rosen (EPR) entanglement—that quantifies the possibility of acquiring some knowledge about the quantum nature of the state of one beam by measuring the other one. For twin beams, the conditional variances $V_{X_a|X_b}$ and $V_{Y_a|Y_b}$ are smaller than $2\langle\Delta\hat{X}_-^2\rangle$ and $2\langle\Delta\hat{Y}_+^2\rangle$, respectively (*29*). As a consequence, with our experimental results, $V_{X_a|X_b} \cdot V_{Y_a|Y_b} \leq 4\langle\Delta\hat{X}_-^2\rangle\langle\Delta\hat{Y}_+^2\rangle = 0.55 < 1$, which gives an upper bound for the EPR criteria and is a sufficient condition for the presence of EPR entanglement.

To fulfill the strict criteria of relative-phase stability, we found it convenient to generate the LOs by using the very 4WM process under study, in the spirit of Kim *et al.* (*30*). As explicitly shown in Fig. 3B, the pump beam is equally split into two parts before the vapor cell. One part is used to generate the vacuum twin beams, and the other part is mixed with a probe seed to generate two bright twin beams that we use as LOs. Several points concerning this method deserve discussion. First, all the beams are derived from the same laser and are therefore relative-phase stable. No active phase stabilization is required. Second, the LOs themselves display multi–spatial-mode entanglement, but this has a negligible impact on the measured entanglement of the vacuum twin beams in the limit of well balanced HDs (*23*). Third, the vacuum modes that are analyzed in the HDs are those that completely overlap with the bright part of the LOs. Fourth, the seeded 4WM process produces bright twin beams with slightly unbalanced intensities, resulting in HDs with unequal optical gains. We can correct for this by adjusting the electronic gain of one of the balanced detectors, but at the level of squeezing that we observe and at our operating gain we found that this has little impact. Lastly, the major advantage of the method is that the analyzed spatial modes are automatically matched, because the LOs themselves are generated in a gain medium having the same characteristics as the one used to generate the vacuum twin beams, such as the Kerr-lensing effect. This feature is particularly important when analyzing multi–spatial-mode squeezed light, because any mismatch between the modes selected by the HDs leads to the partial detection of noisy and uncorrelated optical modes. This adds excess noise to the signal and can bring the recorded noise well above the QNL, as has already been observed for intensity-difference squeezing (*25*). This is in contrast to the case where only a single spatial mode experiences gain. In that case, mode mismatch in the HDs merely results in a loss of detection efficiency, which leads only to a reduced level of measured squeezing. The effect of the excess noise in the imperfectly mode-matched HDs might explain why our best measured squeezing occurs at a rather low amplifier gain.

The multi–spatial-mode operation of the amplifier is demonstrated by the arbitrariness of the position and angle of the LOs, which only have to be chosen symmetrically with respect to the pump direction and inside the solid angle of acceptance. This point can be made even more clear by putting a mask on the probe beam seeding the LOs, in this case a T. Both LOs then carry a T image, as shown in Fig. 4B. The noise of the joint quadratures is slightly increased to –3.6 dB, with respect to the QNL, while remaining well below the inseparability and the EPR limits. Figure 4B also shows the transverse profile of the LOs in the detection region and the far field for the conjugate LO, highlighting the differential lensing effect experienced by the probe and the conjugate LOs. Of course, the vacuum twin beams are also subject to the same effect and the mode matching between the vacuum modes interrogated by the two LOs occurs naturally. On the other hand, it would be difficult to construct ad hoc LOs with properly matched transverse profiles.

Quadrature entanglement can therefore be demonstrated using as LOs any pair of bright twin images that can be created by the 4WM process. For instance, the generation of bright Laguerre-Gauss twin beams has already been demonstrated in this system (*25*). Because Laguerre-Gauss modes of first order are a superposition of TEM_{10} and TEM_{01} (transverse electromagnetic) modes, this hints at the fact that entanglement should be observed with LOs in the TEM_{10} and TEM_{01} modes. This, in turn, would imply that Gaussian-shaped bright twin beams created by 4WM are entangled in their position and momentum (*31*). In the general case, the quadrature variables of a pair of spatial modes cannot be interpreted as familiar conjugate variables of a pair of bright beams, such as their positions and momenta, but the entanglement of these quadratures corresponds to the most general form of spatial entanglement (*32*).

The analysis of the multi–spatial-mode vacuum twin beams by means of pairs of bright beams used as LOs also sheds some light on the complexity of the spatial entanglement. Such a set of spatially orthogonal pairs of modes would

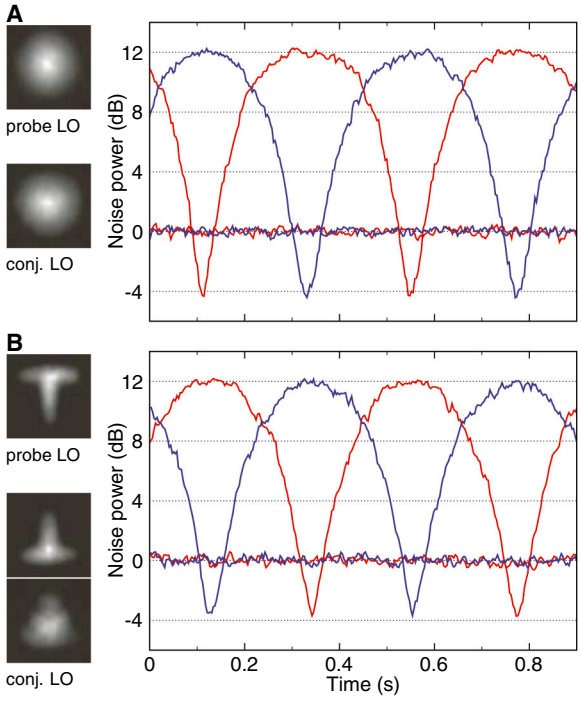

Fig. 4. Quadrature squeezing. The traces show the normalized noise power of the sum (blue) and difference (red) of the quadratures measured by the two HDs, as the phases of the LOs are scanned simultaneously. When the two-mode squeezed vacuum is blocked, the HDs record the QNL, which is set at 0 dB. When the fields emitted by the 4WM are measured, the difference and sum noise curves drop successively below the QNL, revealing the squeezing of both $\hat{X}_-$ and $\hat{Y}_+$. $\langle\Delta\hat{X}_-^2\rangle$ and $\langle\Delta\hat{Y}_+^2\rangle$ correspond to the minima of the red and blue curves, respectively. The images on the left show the intensity profile of the LOs at the position of the HDs. (**A**) Entanglement for Gaussian modes, with –4.3 dB of squeezing on both joint quadratures. (**B**) Entanglement for T-shaped modes. The minimum noise is –3.6 dB for both traces. The conjugate mode is shown both at the position of the HD (lower image) and in the far field (upper image). The LOs appear to have similar spatial modes but not the same focal plane.

correspond to the eigenmodes of the Schmidt decomposition of the biphoton wave function created in the 4WM process. This type of theoretical analysis was carried out for PDC (*33*). A measure of the spatial entanglement is provided by the Schmidt number, which is the "average" number of Schmidt modes involved. A rough estimate of that number is given by the number of gain-medium diffraction-limited Gaussian twin beams that fit into the solid angle of acceptance (*23*), and it naturally matches the previous estimate of the number of independent modes, counted in terms of non-overlapping coherence areas (*25*, *34*). Such a large Schmidt number highlights the potential of this system as a source of high-dimensional entanglement. The exact details of the entanglement, contained in the coefficients of the Schmidt decomposition, depend on the spatial gain profile and can be tuned by varying the spatial mode of the pump laser. A major challenge will be to use that degree of freedom to generate and detect useful multimode states.

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35. R.C.P. is supported under a grant from the Intelligence Community Postdoctoral Program.

Supporting Online Material

www.sciencemag.org/cgi/content/full/1158275/DC1
Materials and Methods
SOM Text
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References

25 March 2008; accepted 4 June 2008
Published online 12 June 2008;
10.1126/science.1158275
Include this information when citing this paper.

Phase Transitions of Dirac Electrons in Bismuth

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The Dirac Hamiltonian, which successfully describes relativistic fermions, applies equally well to electrons in solids with linear energy dispersion, for example, in bismuth and graphene. A characteristic of these materials is that a magnetic field less than 10 tesla suffices to force the Dirac electrons into the lowest Landau level, with resultant strong enhancement of the Coulomb interaction energy. Moreover, the Dirac electrons usually come with multiple flavors or valley degeneracy. These ingredients favor transitions to a collective state with novel quantum properties in large field. By using torque magnetometry, we have investigated the magnetization of bismuth to fields of 31 tesla. We report the observation of sharp field-induced phase transitions into a state with striking magnetic anisotropy, consistent with the breaking of the threefold valley degeneracy.

The Dirac Hamiltonian, long the accepted theory of relativistic fermions, is equally successful in describing electrons in solids, notably bismuth (*1*–*3*), $Bi_{1-x}Sb_x$ (*4*), and graphene (*5*, *6*). Unlike in regular solids, the electron energy, $E(\mathbf{p})$, in these materials is linear in the momentum, $\mathbf{p}$, just as in relativistic fermions. However, Dirac electrons living in solids have two distinguishing features. First, because of their small (or zero) mass gap, the Dirac bands become quantized into Landau levels in a modest magnetic field, $\mathbf{H}$. A striking consequence in graphene is the observation of the integer quantum Hall effect (QHE) (*5*, *6*). The Coulomb interaction energy is crucially important when all electrons are confined to the lowest Landau level. Secondly, the Dirac electrons in solids come in different "flavors," corresponding to orbital valley degeneracy. The interplay of strong interaction and degeneracy suggests that, in intense $\mathbf{H}$, a phase transition may occur to a collective state with novel quantum properties (*7*). In bismuth, the Dirac electrons occupy three Fermi surface (FS) ellipsoids. By using a torque cantilever to measure its magnetization, we have observed sharp field-induced transitions. The high-field ordered state is magnetically anisotropic, consistent with the breaking of the flavor (valley) degeneracy.

In bismuth, the hole FS ellipsoid is aligned with the trigonal axis $\mathbf{z}$ (*8*) (Fig. 1A, inset). The three-electron FS ellipsoids, arrayed symmetrically around the hole FS, are tilted by a small angle β (~6.5°) out of the plane defined by the bisectrix ($\mathbf{x}$) and binary ($\mathbf{y}$) axes. Extensive studies have established that the electron ellipsoids have a Dirac dispersion (*1*, *2*, *9*, *10*). In the geometry with $\mathbf{H}\|\mathbf{z}$, each Landau level of the electrons has a threefold valley degeneracy. However, the near-equality of the hole and electron FS areas (projected onto the *xy* plane) has long stymied efforts to resolve the quantum oscillations of the electrons from the holes (*11*–*14*). This roadblock has to be overcome before the Dirac electrons can be investigated. We solved this problem with torque magnetometry (*8*) [section I of supporting online material (SOM)]. Two sets of data were taken to maximum H =14 T and to 31 T (at temperatures of 1.5 and 0.3 mK, respectively) on single crystals in which the resistance ratio (between 300 K and 4 K) RRR is ~100.

In an increasing $\mathbf{H}$, the Landau level energies rise. As each sublevel crosses the chemical potential μ and empties, a break-in-

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slope appears in the free energy F versus H (eq. S5 of SOM). We assume **H** is at an angle θ to a FS symmetry axis $\hat{\mathbf{e}}$. In terms of the area $S(\theta)$ of the FS section normal to **H**, the breaks occur at the fields $B_n(\theta)$ given by

$$\frac{1}{B_n} = \frac{2\pi e}{\hbar}[n+\gamma]\frac{1}{S(\theta)}, \ (n = 0, 1, 2,...) \quad (1)$$

where e is the electron charge, $2\pi\hbar$ is Planck's constant, and γ the Onsager phase. We label the sublevels as (n, s), where $s = \pm 1$ indexes the Kramers doublet.

In the tilted **H**, a torque, **τ**, appears with magnitude given by $\tau = -\partial F/\partial\theta$. The torque arises because the orbital, diamagnetic moment **m** tends to align with $\hat{\mathbf{e}}$ if $\theta << 1$. Hence, the Landau eve crossings may be observed as a series of sharp anomalies in a trace of τ versus H (fig. S1). In addition to the high-field oscillations, the torque also senses a featureless "background" term $\Delta\chi_b$ arising from the unusually large diamagnetic susceptibility (3).

The symmetry between the three ellipsoids is broken by tilting **H** in the yz plane (Fig. 1A inset). Because the cantilever by design responds to the x component of **τ** only, it is sensitive to the torques from ellipsoids 2 and 3 and less so to the holes (ellipsoid 3 is nearly invisible; see eq. S16 of SOM).

We expressed the torque signal τ as a transverse magnetization, viz. $M_T = \tau/VH$, with V the sample volume. In Fig. 1, curves of M_T versus H are displayed with θ confined to the narrow window (–4°, 4°) in which the transitions occur. As shown, $M_T(H)$ contains a dense set of quantum oscillations, which mostly arise from the electron FS. At large H, we observed sharp, hysteretic jumps at a field H_2 that shifts rapidly with θ. As θ increases from –3.5° to –0.3° (Fig. 1A), the transition field H_2 (black arrows) increases from 14 to 24 T. For $\theta > 0$ (Fig. 1B), H_2 falls back to 13 T as $H_2 \rightarrow 2.1°$. In addition, a transition occurs at the lower field H_1 (red arrows).

First, we identified the electron quantum oscillations by using the derivatives $\partial M_T/\partial H$ in the low-field set. Figure 2, A and B, shows curves measured at θ = –3.1° and 3.4°, respectively. As indicated, the index fields $B_{n,s}$ appear as sharp minima for $\theta < 0$ and as sharp maxima for $\theta > 0$ (fig. S3). When displayed in the θ – H plane, the index fields $B_{n,s}$ describe a nominally symmetric pattern (Fig. 2C). The set of 19 angles investigated and the smooth variations versus θ allow us to sort out most of the sublevels. By Eq. 1, the θ dependence of $B_{n,s}$ reflects the angular dependence of S in ellipsoids 2 and 3. Fields $B_{n,s}$ that increase with increasing θ arise from 2, and those that decrease with increasing θ arise from 3. At large H, the sign of τ provides further guidance. When a sublevel (n, s) crosses the symmetry line θ = 0, its contribution to τ changes sign. The changes (1, –) → (1′, –) and (2, ±) → (2′, ±) are indicated in Fig. 2, A and B (the high-field partner is primed).

A plot of $1/B_{n,\pm}$ versus the integers n (at a fixed θ) gives a straight line with a slope corresponding to an electron FS area $S_e = 6.21$ T (fig. S6). The large number of oscillations (n = 0, · · ·, 10) allows a test of the Dirac spectrum (*1*–*3*) compared with the conventional spectrum. Our measurements agree well with the former but are incompatible with the latter in the limit $n \rightarrow 0$ (section IV of SOM). Hence, the Dirac spectrum underlies the sublevel indexing shown in Figs. 2 and 3.

In Fig. 2C, the lower transition field $H_1(\theta)$ stands out because its dispersion is anomalous (open circles). In contrast to the indexed sublevels, $H_1(\theta)$ displays a hull-shaped bottom with sides rising steeply as $|\theta| \rightarrow 4°$ (fig. S5). Remarkably, within the region shaded white, the curve of the sublevel (0, –), which intersects H_1, loses its intensity abruptly, as discussed below.

Extending the analysis to 31 T, we can map out the high-field phase diagram. In Fig. 3, the white area is now apparent as a cone-shaped region bounded by H_1 from below and by H_2 from above (black curves). As $|\theta| \rightarrow 4°$, the two curves meet at the field ~ 13 T. From Fig. 1A we see that, in curves with $\theta > 0$, M_T displays a step downward (red arrow) as we enter this region, followed by a step upward (black arrow) as we exit. The sublevel (0, –) line vanishes on entering this region (fig. S5). At angles $|\theta| > 4°$, it reappears outside the cone region, rising to 17.5 T at θ = 11° (blue circles). Within the cone region, the electronic state is distinct from that outside.

This difference is apparent if we make H-constant cuts of the transverse magnetization $M_T(\theta, H)$ versus θ. Figure 4A shows curves at several H values increasing from 10 to 31 T in 1-T steps (curves are displaced by 10 A/m for clarity). All the curves display a "background" term that is θ-linear with a nominally H-independent slope. By its sign, we may identify it with the anisotropy $\Delta\chi_b = \chi_z - \chi_y$ of the unusually large diamagnetic susceptibility (*3*).

At the lower field values (10 to 14 T), M_T exhibits jumps when θ crosses the curve of H_1 (shown as red circles). These are the jumps indicated by the red arrows in Fig. 1. As H rises

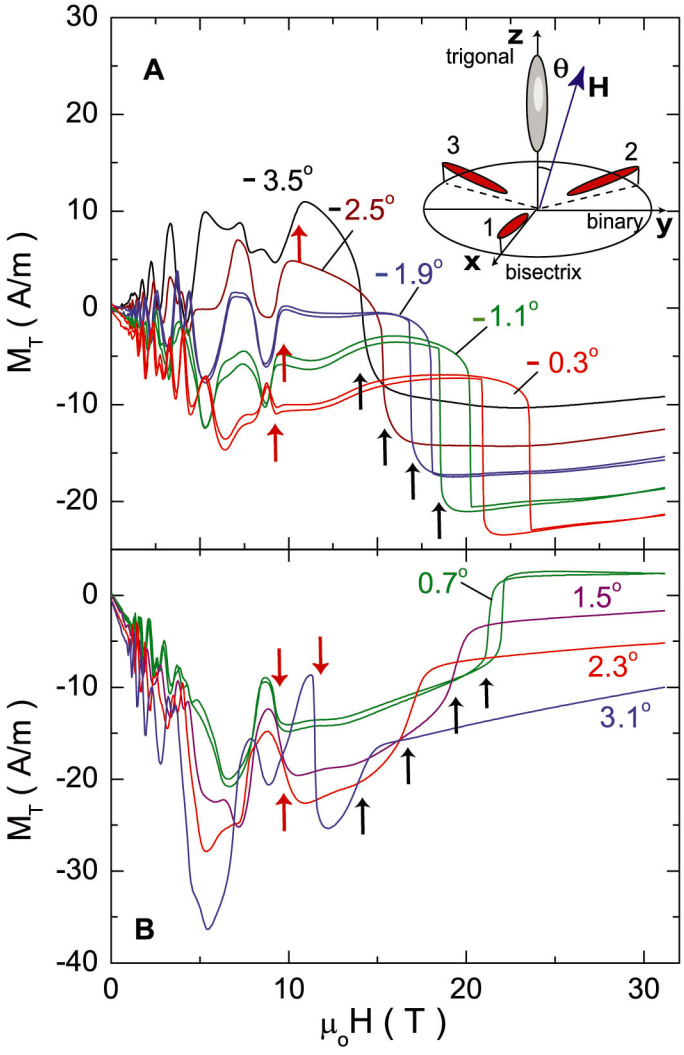

Fig. 1. The H dependence of the torque signal displayed as $M_T = \tau/VH$, with **H** at angles $|\theta| < 4°$. (**A**) and (**B**) show curves with $\theta < 0$ and $\theta > 0$, respectively. In each curve, electronic transitions are observed at H_1 (red arrows) and at H_2 (black arrows). [(A) inset] The hole (gray) and electron (red) FS ellipsoids. **H** lies in the yz plane. The sign of M_T is that of $\tau \cdot \mathbf{x}$. μ_0 is the vacuum permeability.

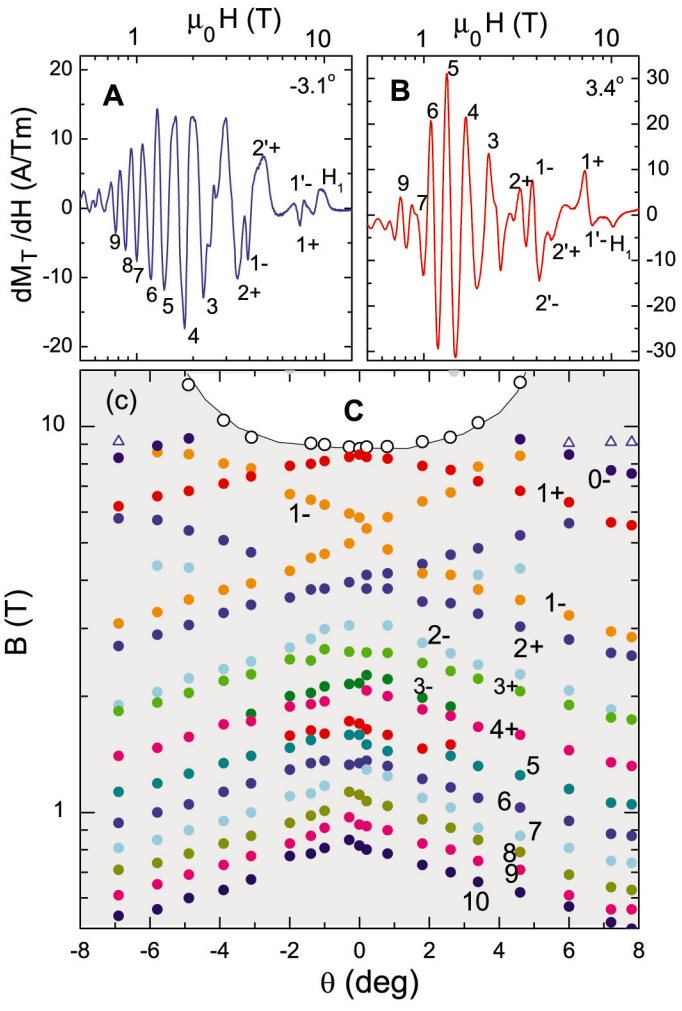

Fig. 2. Traces of the derivative dM_T/dH versus $H = |\mathbf{H}|$ at $\theta = -3.1°$ and 3.4° [(**A**) and (**B**), respectively] at 1.5 K. The sublevels (n, s) are identified by minima for $\theta < 0$, and sublevels $(s = \pm)$ are identified by maxima for $\theta > 0$. Primed labels, for example, $(2', +)$, indicate sublevels that have crossed the line $\theta = 0$. At low H, the label $(n, +)$ is shortened to n. (**C**) The dispersion of sublevels (n, s) versus θ in the θ-H plane with H in log scale. As θ increases, sublevels with $B_{n,s}$ decreasing belong to ellipsoid 2, and sublevels with $B_{n,s}$ increasing belong to ellipsoid 3. At large H, the sublevels $(1, \pm)$ and $(2, \pm)$ may be followed over a broad range of θ. The hole sublevel $n = 1$ is the θ-independent curve at 9.2 T (triangles). The curve H_1 (open circles) is the lower boundary of a new state.

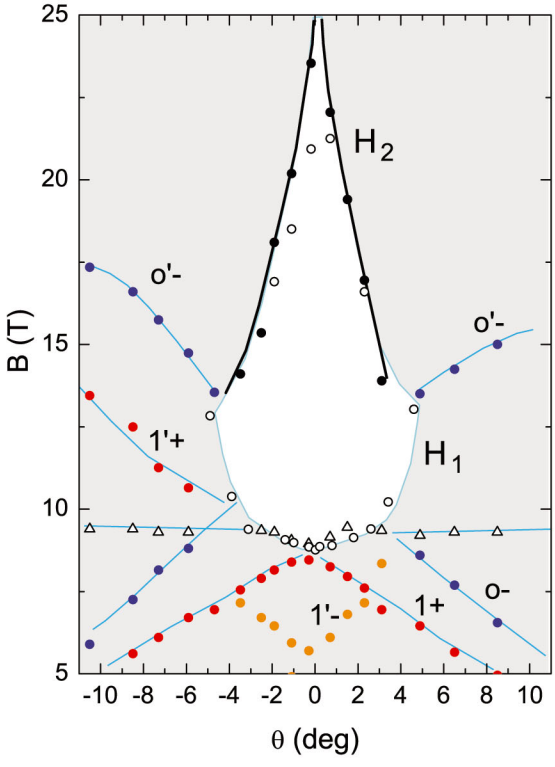

Fig. 3. High-field phase diagram in the B-θ plane (T = 0.3 K). The two transition fields H_2 (red circles) and H_1 (open circles) enclose a region (white) in which the torque signal vanishes (apart from the background $\Delta\chi_b$). The sublevel (0,–) vanishes in amplitude when it enters this region but reappears at larger tilt $|\theta|$. The horizontal line at 9.3 T is the n = 1 sublevel of the hole ellipsoid.

to values 14 to 25 T, the jumps occur when θ crosses H_2 (black circles). With increasing H, the interval bounded by the curve $H_2(\theta)$ steadily shrinks, until it falls below our angular spacing $\delta\theta \sim 1°$ above 25 T.

A striking feature is the uniform topography of $M_T(\theta, H)$ throughout the cone region. This is emphasized in Fig. 4B, which replots the low-field curves without displacing them. Within the cone, $M_T(\theta, H)$ stays independent of H despite a 40% increase in H. Identifying the common slope of M_T versus θ with the background term $\Delta\chi_b$, we infer that there is no further contribution to the torque in the quantum limit; the high-field torque is clamped at zero inside the cone region. This accounts for the vanishing of the signal from (0, –) as its trajectory enters the cone region. When we leave this region, by changing either H or θ, a finite high-field torque reappears as a jump discontinuity at the boundaries H_1 and H_2. The first-order nature of the jumps and the finite angular width of the cone region suggest that, as H is tilted slightly from **z**, the system persists in the zero-torque state. The steep decrease of $H_2(\theta)$ with increasing $|\theta|$ also emphasizes the importance of the threefold degeneracy to the zero-torque state. A slight tilt of **H** allows the state to be destabilized at a lower H_2.

The state above the curve of $H_2(\theta)$ is also unexpected (Fig. 4A). In constant-H cuts at large H, $M_T(\theta)$ undergoes a finite jump at $\theta = 0$, abruptly reaching a nominally constant value on both sides. Ignoring the background slope, we may express the profile as $M_T = \mathrm{sgn}(\theta)M_0$, with M_0 independent of θ and **H**. This profile implies saturation of the transverse magnetization, as well as an abrupt sign change at $\theta = 0$. If viewed as a plot of M_T versus H_y, it is suggestive of a ferromagnetic response (but not involving the physical spins). By contrast, M_T at $n = 0$ for noninteracting electrons does not saturate (see eq. S13 of SOM). Both the saturation and sign change imply that the noninteracting Landau level scheme is inadequate in high fields.

The problem of interacting electrons with several valley degrees is also of importance in the 2D (two-dimensional) QHE systems based on bilayer GaAs (*7*, *15*, *16*) and graphene (*17*–*19*). In the large-H limit, the electrons gain exchange energy if they all occupy the same valley (or a linear combination of valleys). This state, involving "valley polarization," is called valley ferromagnetism. In bismuth (with $\mathbf{H}\|\mathbf{z}$), the presence of a threefold valley degeneracy enriches the problem in a novel way; the system may either form a symmetric combination of the three valleys or occupy two of them. The magnetic anisotropy and ferromagnetic-like profile revealed in Fig. 4 strongly suggest a valley-polarized state.

Our results confirm that, in bismuth, the Dirac electrons with three flavors are unstable to the formation of a novel ground state in intense mag-

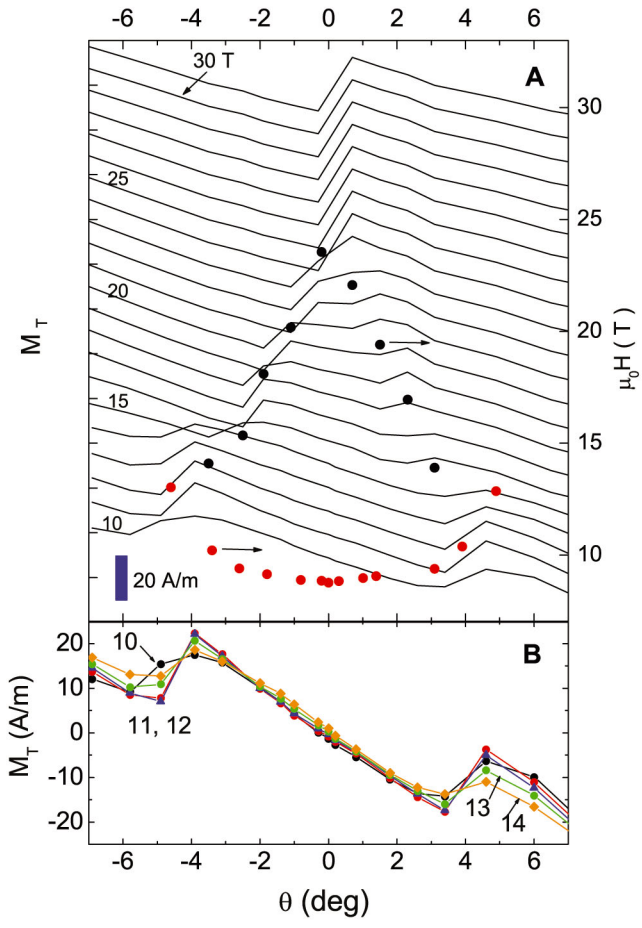

Fig. 4. Constant-field cuts of the transverse magnetization $M_T(\theta, H)$. (**A**) Cuts for $10 < H < 31$ T in 1-T steps, displaced by 10 A/m between adjacent curves (blue bar indicates $\Delta M_T = 20$ A/m). Transitions occur when θ crosses the curves H_1 (red circles) and H_2 (black circles). The background tilt arises from the susceptibility anisotropy $\Delta\chi_b$. At high fields ($H > 25$ T), M_T retains its discontinuity at $\theta = 0$. (**B**) Cuts at 10 to 14 T without displacement. In the angular window $|\theta| < 4°$, M_T is independent of H.

netic fields. The 3D electronic state in bismuth is easier to probe with thermodynamic and spectroscopic tools and hence promising for elucidating the high-field instability of the Dirac gas.

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Supporting Online Material

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9 April 2008; accepted 9 June 2008
10.1126/science.1158908

Did Cooling Oceans Trigger Ordovician Biodiversification? Evidence from Conodont Thermometry

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The Ordovician Period, long considered a supergreenhouse state, saw one of the greatest radiations of life in Earth's history. Previous temperature estimates of up to ~70°C have spawned controversial speculation that the oxygen isotopic composition of seawater must have evolved over geological time. We present a very different global climate record determined by ion microprobe oxygen isotope analyses of Early Ordovician–Silurian conodonts. This record shows a steady cooling trend through the Early Ordovician reaching modern equatorial temperatures that were sustained throughout the Middle and Late Ordovician. This favorable climate regime implies not only that the oxygen isotopic composition of Ordovician seawater was similar to that of today, but also that climate played an overarching role in promoting the unprecedented increases in biodiversity that characterized this period.

Oxygen isotopes provide a valuable means for tracking environmental change, with oxygen isotopic compositions of marine fossil carbonates ($\delta^{18}O_{carb}$) being especially useful for reconstructing Cenozoic sea-surface temperatures (*1*). This approach, however, has proven less reliable for older biogenic carbonates, which are more prone to diagenetic modification. Anomalously low oxygen isotope compositions of Early Paleozoic biogenic carbonates have driven contentious debate about the implied high seawater temperatures and variability in seawater oxygen isotopic composition ($\delta^{18}O_{seawater}$). Resolution of these issues has important implications for understanding fundamental Earth processes and major events in Earth history.

The Paleozoic marine $\delta^{18}O_{carb}$ record is derived mainly from calcitic brachiopods (*2*, *3*) that exhibit a wide range in composition from about −2 per mil (‰) to −10‰ Vienna Pee Dee belemnite (V-PDB). It has been argued that this re-

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cord reflects secular variations in global climate (*2*, *4*) but, assuming present-day values for seawater ($\delta^{18}O_{seawater} \sim -1$), the calculated temperatures are unrealistically high, reaching 70°C in the Early Ordovician. This seeded further speculation that $\delta^{18}O_{seawater}$ composition, and by implication hydrothermal processes, evolved with time (*3*, *5*–*7*). This is due to the inherent limitation of the oxygen isotope proxy being a combined signature of temperature and seawater isotopic compositions, thereby comprising two unknowns. The proposition that $\delta^{18}O_{seawater}$ changed significantly over time is, however, inconsistent with altered seafloor basalt compositions and models of the seawater isotopic budget, which imply that $\delta^{18}O_{seawater}$ has remained essentially constant since the Archean (*8*–*11*). This suggests that these older biogenic carbonates do not reflect primary paleoseawater compositions, yet these issues remain contentious.

The temperature record of Ordovician oceans is central to understanding links between seawater chemistry, climate change, major bio-events, and thus fundamental Earth processes. The marine biosphere underwent a profound transformation during the Great Ordovician Biodiversification Event (GOBE), recognized as the longest period of sustained biodiversifications, increasing family and genus numbers three- to fourfold (*12*) (Fig. 1). This unparalleled event is characterized by the replacement of the Cambrian Evolutionary Fauna with considerably more complex Paleozoic and Modern Evolutionary Faunas. The GOBE was terminated with sudden and catastrophic extinctions during the latest Ordovician (Hirnantian), probably associated with rapid ice sheet growth at the South Paleopole (*13*).

Although there are different regional biodiversification patterns, which are well recognized from biotal provincialism, at a higher level there are important global trends. Major increases in biocomplexity and biodiversity began in the Early Ordovician with notable expansions throughout the pelagic realm. The onset of the first major biodiversity surge occurred during the Mid Ordovician with extensive colonization of the benthos including the establishment of hardgrounds and reefal systems (*14*), although diversifications of many groups peaked throughout the Late Ordovician (*12*, *14*, *15*). The causal mechanism(s) that drove these radiations has been elusive and enigmatic given the long-standing belief that supergreenhouse conditions prevailed. Clearly, local and regional environmental conditions (e.g., sedimentation, eustasy, temperatures, nutrients, ocean circulation) would have substantially shaped the character of evolving marine communities, as would various biophysical mechanisms. However, overarching global conditions (e.g., climate, sea level) would have also played key roles in this major reconfiguration of the marine biosphere.

To better characterize the Ordovician climate regime, we have used conodont apatite as a potentially robust temperature archive. Although not as abundant or easy to analyze as carbonate, the phosphate mineralogy of conodont microfossils is more stable than that of biogenic marine carbonates. Furthermore, conodonts are ubiquitous in Cambrian-Triassic marine sequences worldwide and evolved rapidly, providing fine stratigraphic resolution. A major drawback, however, is that conodonts are small (~0.1 to 3 mm long), so previous analyses (*16*, *17*) have typically required "bulk" sampling, even those using infrared laser isotope ratio monitoring gas chromatographic mass spectrometry (*18*). This is less than ideal because such samples may contain contaminants and remnant basal tissue that can compromise the analysis (*18*). Moreover, compositional heterogeneity between different taxa will not be discernible by bulk analyses, thereby requiring analysis of monospecific samples that are rarely available in sufficient volume. Bulk analyses of Silurian-Carboniferous conodonts have nonetheless yielded oxygen isotope compositions [$\delta^{18}O_{phos}$ = ~18 to 23‰ Vienna standard mean ocean water (V-SMOW)] (*19*) giving plausible paleotemperatures (~33° to 17°C) (*16*–*18*, *20*, *21*), encouraging further exploration of the technique.

Here we show the feasibility of in situ oxygen isotope analysis of single conodont elements at a 30-μm scale using the SHRIMP II ion microprobe at The Australian National University (ANU), recently configured for high-precision stable-isotope analysis (*22*). Durango apatite was used as the primary isotope standard, its composition independently determined by gas isotope ratio mass spectrometry ($\delta^{18}O_{apatite}$ = 9.4‰). The tooth enameloid of a modern great white shark ($\delta^{18}O_{apatite}$ = 22.3‰) was used as a secondary standard. The standard deviation of replicate analyses of these standards ranged from 0.42 to 0.14‰ within sessions, with higher precision reflecting ongoing technical improvements. The conodonts (179 analyses, 102 specimens) were sampled from 20 Ordovician to Early Silurian temporal horizons from 8 sites of similar tropical paleolatitudes (*23*) across Gondwana (Australia) and Laurentia (Canada), of primarily shallow subtidal (with two distal slope) facies (*22*). Consistent shallow-water sampling avoided isotopic variability that would likely be expressed by mixed biofacies. Mean population compositions (Fig. 2) were determined with a precision of typically 0.5‰ (95% confidence level). At this precision, no pronounced isotopic differences were found between tissue types (hyaline or albid) within individual conodont elements, or between most elements within a single sample population. A larger range of compositions was found in some Hirnantian conodont populations, and in an older sample (Manitoba) possibly affected by hypersaline conditions.

The measured conodont $\delta^{18}O_{apatite}$ compositions are internally consistent across geographically disparate sites from two cratons, with no systematic patterns related to facies, and are thereby interpreted as a global temporal trend (Fig. 2). Furthermore, the earliest Ordovician conodonts from Australia have compositions equivalent to those from Texas reported by Bassett *et al.* (*24*), and the Wenlock data from Cornwallis Island are consistent with coeval samples from Gotland (*18*). Throughout the Ordovician, $\delta^{18}O_{apatite}$ increased from ~15.3 to ~19.6‰ (V-SMOW), equivalent to temperatures from ~42° to ~23°C ($\delta^{18}O_{seawater} = -1$). Our $\delta^{18}O_{apatite}$ record provides a first-order primary trend of Ordovician climate variability that discriminates four main climate regimes: (i) sustained cooling during the Early Ordovician (~15.3 to ~18.3‰), reflecting a global shift from greenhouse conditions (~42°C) to modern equatorial temperatures (~28°C) over ~25 million years (Myr); (ii) climate stability with modern equatorial temperatures for ~20 Myr from the Mid through Late Ordovician; (iii) a rapid temperature drop (*25*) during the Hirnantian, marking the well-known latest Ordovician glaciation; and (iv) a return to modern equatorial temperatures by the early Wenlock (Fig. 3). Higher–

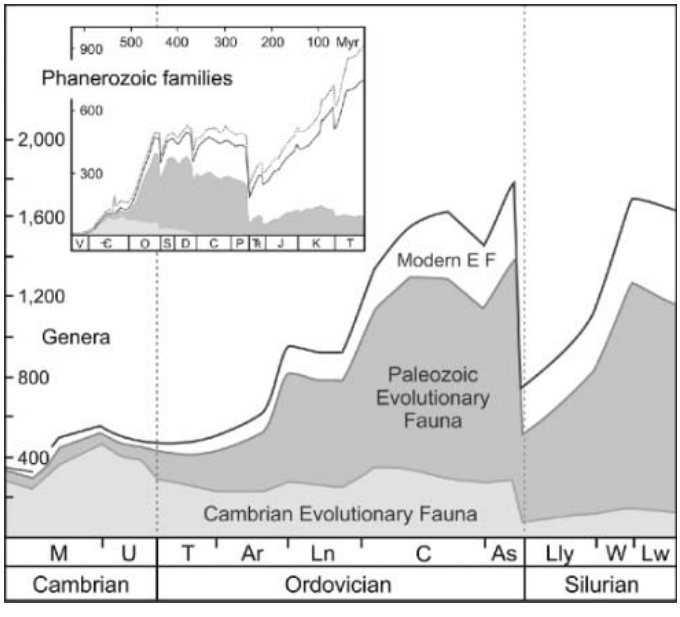

Fig. 1. Biodiversity patterns of marine fauna through geological time. Middle Cambrian to Silurian (except Pridoli) taxonomic diversity trends at genus level [modified from Sepkoski (*15*)]. Inset shows Phanerozoic taxonomic diversity of marine faunas at family level [modified from Sepkoski (*12*)]. Cambrian: M, Middle; U, Upper. Ordovician T, Tremadoc; Ar, Arenig; Ln, Llanvirn; C, Caradoc; As, Ashgill. Silurian: Lly, Llandovery; W, Wenlock; Lw, Ludlow.

temporal resolution studies will be required to characterize finer-scale climate variability throughout this period.

This conodont $\delta^{18}O_{apatite}$ record (Fig. 2) indicates that sea-surface temperatures for much of the Ordovician to Early Silurian were well within present-day ranges (Fig. 3). Even our highest temperature estimate (~42°C), when considering analytical (~3°C range) as well as $\delta^{18}O_{seawater}$ uncertainties, is essentially at the upper limit of present-day surface waters (e.g., Red Sea, Persian Gulf, Sunda Sea). This is consistent with suggestions that the Late Cambrian to Early Ordovician was a higher-temperature interval that favored microbial buildups and exceeded the tolerance of many stenothermal reef-associated organisms (*26*). The Ordovician $\delta^{18}O_{apatite}$ record, however, contrasts markedly with earlier perceptions of a predominantly "supergreenhouse" state, and the carbonate record (*2*, *3*) of considerably lower and dispersed $\delta^{18}O_{carb}$ values, which implies that those samples were dominated by secondary alteration processes. This negates arguments that hydrothermal alteration processes controlling $\delta^{18}O_{seawater}$ have changed appreciably over time. Furthermore, our conodont climate record is inconsistent with the Early Paleozoic "icehouse" and "greenhouse" modes inferred from the detrended $\delta^{18}O_{carb}$ record (*4*), which are exaggerated and temporally expanded. Collectively, the restricted range in $\delta^{18}O_{seawater}$ (±2‰) evidenced by seafloor basalt compositions, and modern-like values ($\delta^{18}O_{seawater} = -1$) suggested by conodont apatite and carbonate clumped-isotope thermometry (*27*), show that there is no physical basis for linearly detrending the $\delta^{18}O_{carb}$ record, thereby invalidating models founded on that approach (*4*, *28*).

The Early to Mid Ordovician cooling phase reflects an important change in Early Paleozoic climate, which likely played a key role in determining marine biodiversity patterns (Fig. 3). Perhaps most notable was the widespread biotic colonization of the planktonic realm from a limited benthic mode, as illustrated by the evolution of Cambrian dendroid graptolites to Ordovician planktonic anisograptids (*29*), with a similar pattern expressed by radiolarians (*30*). It has also been proposed that planktotrophy may have evolved during the Early Ordovician in response to pressures of benthic predation (*31*). The clear paleontological evidence of increased biomass throughout the water column and consequent increased carbon burial, an important controller of atmospheric CO_2 concentration, probably contributed to this cooling trend.

The cessation of cooling and stabilization at modern equatorial temperatures indicates that a new equilibrium between partial pressure of CO_2 (pCO_2) and biological productivity had been reached by the Mid Ordovician. This new moderate climate regime likely spurred major biodiversifications by providing more favorable conditions consistent with modern-day equatorial temperatures (Fig. 3). Expansions were especially prolific in the benthic realm, including new complex metazoan-algal reefal communities and the circumglobal establishment of coral reefs by the mid Late Ordovician (*14*). Such widespread carbonate biomineralization possibly reflected increased seawater carbonate saturation due to decreasing pCO_2 (*32*), and a cooler moderate climate regime consistent with the thermal tolerance window of modern corals. This plausible scenario contrasts with recent suggestions that asteroid impacts triggered the Mid to Late Ordovician biodiversifications (*33*), which offer no credible mechanisms and are typically associated with extinction events.

The Late Ordovician temperature fall in the $\delta^{18}O_{apatite}$ record coincides with the Hirnantian glaciation (time slice 6c; Fig. 3), but suggests a considerably smaller shift on the order of ~1‰ (~4°C), compared to the ~4‰ increase determined from biogenic carbonates from Baltica (*34*). Either our sampling through this event is incomplete, or the Baltic record is affected by local aberrations and/or diagenesis (*35*). The actual temperature range, however, is difficult to ascertain given that the ice-volume component, which was clearly substantial during this interval, cannot be discriminated (*36*). Notably, the Hirnantian conodont populations tend to have larger ranges in isotopic compositions between specimens (*22*), as confirmed by replicate analyses. Such variations may be attributable to any combination of factors including "vital effects," seasonal variations, and analytical error. Further analyses at high temporal resolution are required to better constrain the duration and magnitude of this event, although the current data are inconsistent with a

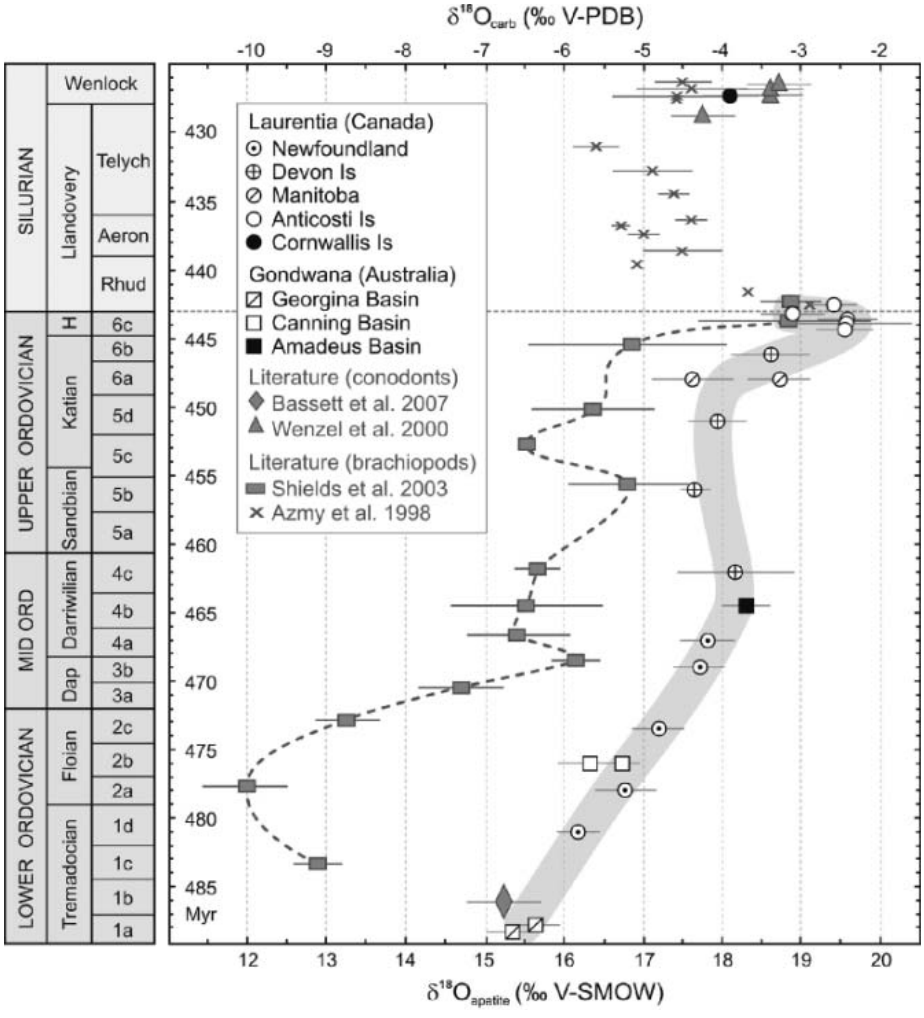

Fig. 2. Oxygen isotope compositions of Ordovician to Early Silurian conodonts and brachiopods. Ordovician and Silurian conodont bioapatite compositions measured during this study using SHRIMP II are compared with compositions previously reported from conodonts (*18*, *24*) and calcitic brachiopods (*3*, *6*). Conodonts analyzed using SHRIMP II are from 20 stratigraphic horizons at 8 different sites across Canada and Australia. Ordovician time scale from Webby *et al*. (*14*) with new stage names from the International Subcommission on Ordovician Stratigraphy; Dap, Dapingian; H, Hirnantian; Rhud, Rhuddanian; Aeron, Aeronian; Telych, Telychian. Error bars for Ordovician brachiopod data (*3*, *6*) are ±1σ (gray squares and crosses), all other data are shown at ±2σ; errors for some means were unavailable; Bassett *et al*. 2007 (*24*) data normalized to NBS 120c = 21.7‰ V-SMOW to allow comparison with our data. Gray band represents the primary first-order temporal trend of Ordovician $\delta^{18}O_{apatite}$ determined by this study.

protracted cooling event (*37*), and clumped isotope thermometry could help constrain ice-volume effects. The dramatic extinctions (the second-largest in Phanerozoic history) that resulted from this rapid glaciation might partly reflect the lack of adaptability of Ordovician biota not previously subjected to such low temperatures.

Although few, the Early Silurian data are consistent with the Mid to Late Ordovician ranges (Figs. 2 and 3), with the transient low Rhuddanian temperature (~24°C) implying climate instability before returning to modern equatorial conditions in the Wenlock (~30°C). Conodont-derived Silurian temperatures are significantly lower than those based on coeval brachiopods (*6*, *18*), again indicating that the oxygen isotope compositions of the carbonates have been compromised (Fig. 2). Recent clumped-isotope thermometry of slightly older calcitic brachiopods (*27*) suggest somewhat warmer conditions (~35°C) for the Telychian (Fig. 3). The latter approach has the advantage of determining temperatures independent of seawater isotopic composition, as well as estimating the $\delta^{18}O_{seawater}$, but the robustness of this technique has yet to be fully validated.

Our $\delta^{18}O_{apatite}$ record demonstrates both the robustness and benefits of high–spatial resolution in situ oxygen isotope analysis of discrete conodont elements, with continued technical advances promising even higher precision and accuracy. Our new global climate record suggests that Early Ordovician "greenhouse" temperatures cooled to present-day conditions that characterized the remainder of the period, suggesting that $\delta^{18}O_{seawater}$ has remained essentially invariant. Furthermore, climate amelioration is coincident with widespread taxonomic radiations, which marked one of the most important evolutionary developments in Earth history. Although temporally and spatially varied, these expansions in biomass and biodiversity were not only modulated by local environmental controls and inherent biophysical mechanisms, but

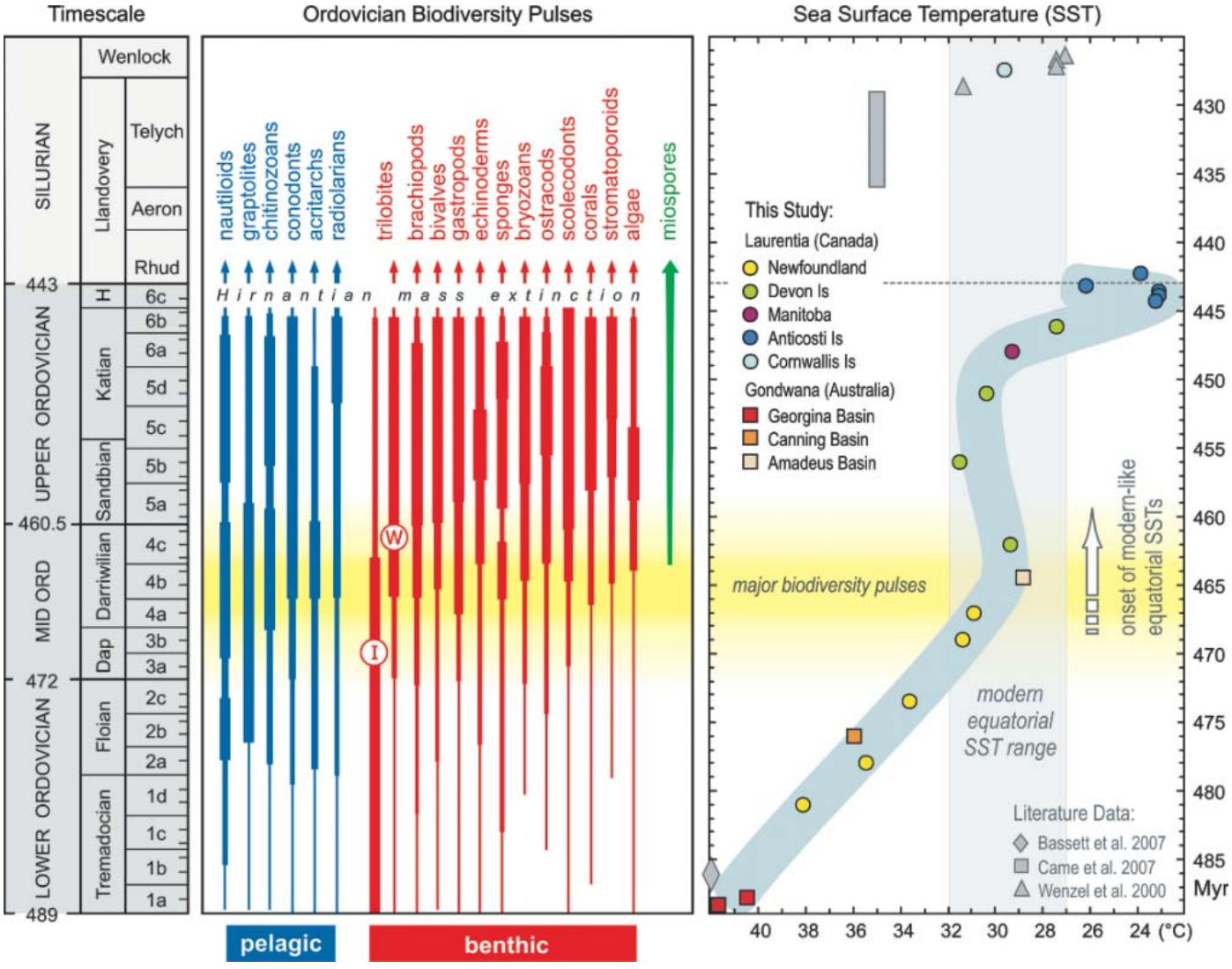

Fig. 3. Generalized global biodiversity pulses and tropical seawater temperature trend through the Ordovician. Temperatures derived from conodont oxygen isotope compositions measured in situ using SHRIMP II show a unidirectional cooling phase and a period of sustained moderate temperatures. Temperature means are plotted for repeat analyses of Canning Basin and Manitoba samples that were determined during different analytical sessions. Blue trend-line represents the primary first-order temporal trend of Ordovician sea-surface temperatures estimated from this study. Yellow band highlights first moderate temperatures and accompanying major biodiversity pulses. Previously reported conodont data (*18*, *24*) are shown together with Silurian carbonate clumped-isotope data (*27*). Timing of biodiversity pulses shown by thickened vertical lines [compiled from data in Webby *et al*. (*14*)], line thickness not proportional between groups, diversity peaks are not represented and vary regionally. Some trilobites are pelagic; I denotes Ibex Fauna, W denotes Whiterock Fauna; algae comprises calcified reds, greens, and cyclocrinids; graptolites based on Chen *et al*. (*31*) and include new planktonic forms, the Graptoloidea; miospores represent terrestrial flora; temperatures were calculated assuming $\delta^{18}O_{seawater} = -1‰$ V-SMOW, with no adjustment for ice-volume effects for Hirnantian Anticosti Island data, and temperatures of Bassett *et al*. 2007 (*24*) based on per mil values normalized to NBS 120c = 21.7‰ V-SMOW.

were likely initiated by this favorable climate regime.

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Supporting Online Material

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29 January 2008; accepted 17 June 2008
10.1126/science.1155814

Enhancement of Thermoelectric Efficiency in PbTe by Distortion of the Electronic Density of States

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The efficiency of thermoelectric energy converters is limited by the material thermoelectric figure of merit (zT). The recent advances in zT based on nanostructures limiting the phonon heat conduction is nearing a fundamental limit: The thermal conductivity cannot be reduced below the amorphous limit. We explored enhancing the Seebeck coefficient through a distortion of the electronic density of states and report a successful implementation through the use of the thallium impurity levels in lead telluride (PbTe). Such band structure engineering results in a doubling of zT in p-type PbTe to above 1.5 at 773 kelvin. Use of this new physical principle in conjunction with nanostructuring to lower the thermal conductivity could further enhance zT and enable more widespread use of thermoelectric systems.

Thermoelectric (TE) energy conversion is an all-solid-state technology used in heat pumps and electrical power generators. In essence, TE coolers and generators are heat engines thermodynamically similar to conventional vapor power generation or heat pumping cycles, but they use electrons as the working fluid instead of physical gases or liquids. Thus, TE coolers and generators have no moving fluids or moving parts and have the inherent advantages of reliability, silent and vibration-free operation, a very high power density, and the ability to maintain their efficiency in small-scale applications where only a moderate amount of power is needed. In addition, TE power generators directly convert temperature gradients and heat into electrical voltages and power, without the additional need for an electromechanical generator.

All of these properties make them particularly suited for recovering electrical power from otherwise wasted heat, for instance in automotive exhaust systems or solar energy converters. These advantages are partially offset by the relatively low efficiency of commercially available material, limiting the use of the technology to niche applications for the past half century. Recent efforts have focused on nanostructured materials to enhance the TE efficiency.

The efficiency of thermoelectric generators is limited to a fraction of their Carnot efficiency ($\eta_c = \Delta T/T_H$), determined by the dimensionless thermoelectric material figure of merit (*1*), zT:

$$zT = T\frac{S^2\sigma}{\kappa} \qquad (1)$$

where S is the thermoelectric power or Seebeck coefficient of the TE material, σ and κ are the electrical and thermal conductivities, respectively, and T is the absolute temperature. For the past four decades, zT of commercial material has been limited to about 1 in all temperature ranges (*1*).

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Recent progress in TE materials has primarily involved decreasing the denominator of Eq. 1 by creating materials with nanometer-scaled morphology to dramatically lower the thermal conductivity by scattering phonons. Quantum-dot superlattices have reported values of $zT > 2$ (*2*), and silicon nanowires have such a reduced κ that zT approaches that of commercial materials (*3*). Although this certainly provides the evidence that high-zT material can be prepared, the results were obtained on thin films or nanowires that are challenging for high-volume applications that normally rely on bulk materials. Structural complexity on various length scales has successfully reduced κ in bulk TE materials, also yielding $zT > 1$ (*1*, *4*–*8*).

Unfortunately, in bulk material at least, there is a lower limit to the lattice thermal conductivity imposed by wave mechanics: The phonon mean free path cannot become shorter than the interatomic distance (*9*). The minimum thermal conductivity of PbTe is about 0.35 W/mK at 300 K, a value measured on quantum-dot superlattices (*2*). Although lower values have been seen for interfacial heat transfer (*10*), progress beyond this point in bulk materials must come from the numerator of Eq. 1 and in particular the Seebeck coefficient; we describe here a successful approach in this direction for bulk materials.

A strong increase has been predicted in the Seebeck coefficient of nanostructures (*11*, *12*) and was observed experimentally in Bi nanowires (*13*). The basis for the enhancement of S here is the Mahan-Sofo theory (*14*), which suggests the study of systems in which there is a local increase in the density of states (DOS) $g(E)$ over a narrow energy range (E_R), as shown schematically in Fig. 1A. Such a situation can occur when the valence or conduction band of the host semiconductor resonates with one energy level of a localized atom in a semiconductor matrix (*14*). The effect of this local increase in DOS on S is given by the Mott expression (Eq. 2). Here, S depends on the energy derivative of the energy-dependent electrical conductivity $\sigma(E) = n(E)q\mu(E)$ taken at the Fermi energy E_F (*15*), with $n(E) = g(E)f(E)$, the carrier density at the energy level E considered, where $f(E)$ is the Fermi function, q the carrier charge, and $\mu(E)$ the mobility:

$$S = \frac{\pi^2}{3}\frac{k_B}{q}k_B T\left\{\frac{d[\ln(\sigma(E))]}{dE}\right\}_{E=E_F} = \frac{\pi^2}{3}\frac{k_B}{q}k_B T\left\{\frac{1}{n}\frac{dn(E)}{dE} + \frac{1}{\mu}\frac{d\mu(E)}{dE}\right\}_{E=E_F} \quad (2)$$

Equation (2) shows that there are two mechanisms that can increase S: (i) an increased energy-dependence of $\mu(E)$, for instance by a scattering mechanism that strongly depends on the energy of the charge carriers, or (ii) an increased energy-dependence of $n(E)$, for instance by a local increase in $g(E)$. Mechanism (ii) is the basis of the Mahan-Sofo theory, provided that E_F of the semiconductor aligns properly in the range of the excess DOS in the band (Fig. 1A). The concept can also be expressed in terms of effective mass m^*_d, as shown for degenerate semiconductors (*1*):

$$S = \frac{8\pi^2 k_B^2 T}{3qh^2} m_d^* \left(\frac{\pi}{3n}\right)^{2/3} \quad (3)$$

with

$$g(E) = \frac{(m_d^*)^{3/2}\sqrt{2E}}{\hbar^3 \pi^2} \quad (4)$$

Because zT also depends on the carrier's group velocity via the electrical conductivity, the value of E_F that maximizes zT is somewhat different from the value that maximizes S and m^*_d (*14*).

Calculations (*16*) indicate that the group III elements Ga, In, and Tl create additional energy levels, sometimes called resonant levels, in a classical thermoelectric semiconductor, PbTe. We report here that the approach is successful in doubling zT in dilute alloys of PbTe with 1 or 2 atomic % Tl (Tl-PbTe) (Fig. 1B). Review articles have described how the group III elements establish states in the IV-VI compound semiconductors (*17*, *18*). The origin of the Tl-induced states is still under investigation, and they have been ascribed to either a valence fluctuation (*18*) or a hybridization between an excited state of the group III atom and the neighboring Te p-states (*16*), or an additional piece of the Fermi surface (*19*). Considering now all group III atoms in PbTe, we see that the position of the additional energy level is not clear in Ga-PbTe (*17*, *18*), that for In-PbTe it is favorably located in the conduction band at low temperature (*20*) but moves into the energy gap at room temperature (*21*), and that it is favorably located in the valence band of Tl-PbTe (*17*).

Optical measurements suggest that there are several distinct levels associated with Tl in PbTe, one of which is at an energy ~ 0.06 eV below the band edge; the width of such levels depends on the exact composition of the alloy but is on the order of ~0.03 eV (*17*). The strong influence of the Tl level on the valence band of Tl-PbTe is further confirmed by measurements of the electronic specific heat (*22*), which show an increase in the density of available electronic states in the valence band over that of pure PbTe as a function of Tl concentration. An increase by a factor of 2.6 is observed at 1.5 atomic % Tl. The increase is also related to the surprisingly high superconductive transition temperature in the material.

Several disk-shaped samples of $Tl_{0.01}Pb_{0.99}Te$ and $Tl_{0.02}Pb_{0.98}Te$ were prepared (*23*) and mounted for high-temperature measurements (300 to 773 K) of their conductivity (σ and κ), as well as Hall (R_H) and Seebeck (S) coefficients; parallelepipedic samples were cut from the disks and mounted for low-temperature measurements (77 K to 400 K) of galvanomagnetic (ρ and R_H) and thermomagnetic (S and N, which stands for the isothermal transverse Nernst-Ettingshausen coefficient) properties (*23*). The results for the zero-field transport properties measured on representative samples of $Tl_{0.01}Pb_{0.99}Te$ and $Tl_{0.02}Pb_{0.98}Te$ are shown in Fig. 2.

Values of zT for $Tl_{0.02}Pb_{0.98}Te$ reach 1.5 at 773 K (Fig. 1B). The high value of zT observed is quite reproducible and robust with respect to slight variation in dopant concentration in $Tl_{0.02}Pb_{0.98}Te$. The uncertainty in zT is estimated to be on the order of 7% near room temperature and increasing at higher temperature if we assume that the inaccuracies on S, σ and κ are independent of each other (*23*). For the $Tl_{0.01}Pb_{0.99}Te$, the decreased doping levels lead to a lower carrier concentration and a corresponding increase in S and ρ. The values in Fig. 1B represent a 100% improvement of the zT compared with the best conventional p-type PbTe-based alloys ($zT_{max} = 0.71$ for $Na_{0.01}Pb_{0.99}Te$) (*24*). The maximum in zT occurs at the temperature where thermal excitations start creating minority carriers. This maximum is not reached by 773 K for $Tl_{0.02}Pb_{0.98}Te$, and thus, higher values of zT may be expected.

The temperature range where these PbTe-based materials exhibit high zT values (500 to 773 K) is appealing for power generation from waste heat sources such as automobile exhaust.

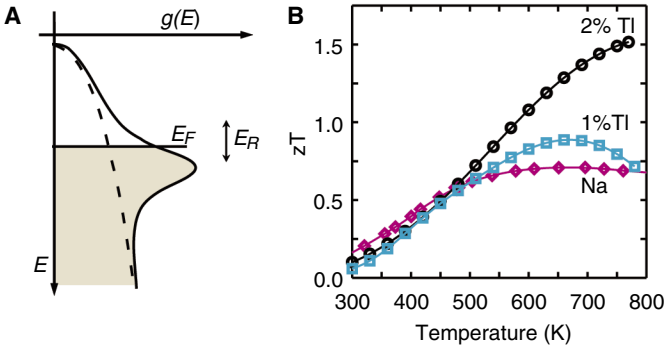

Fig. 1. (**A**) Schematic representation of the density of electron states of the valence band of pure PbTe (dashed line) contrasted to that of Tl-PbTe in which a Tl-related level increases the density of states. The figure of merit zT is optimized when the Fermi energy E_F of the holes in the band falls in the energy range E_R of the distortion. (**B**) The zT values for $Tl_{0.02}Pb_{0.98}Te$ (black squares) and $Tl_{0.01}Pb_{0.99}Te$ (blue circles) compared to that of a reference sample of Na-PbTe (purple diamonds).

Direct thermoelectric efficiency measurements were not conducted because of the nontrivial requirements for a matching *n*-type material, good thermal isolation, and low thermal and electrical contact resistance. The latter consideration arises because the main flow of heat and of electrical current must pass through the contacts of a TE power generator, in contrast to the situation in the experiments reported here.

The κ values of every Tl-PbTe sample measured reproduces that of pure bulk PbTe (*25*). In contrast, all *zT* -enhancing mechanisms used previously in PbTe-based materials have relied on minimizing the lattice thermal conductivity (*1*, *4*, *7*, *8*). The slight rise in κ of the $Tl_{0.02}Pb_{0.98}Te$ sample at high temperatures is attributed to ambipolar thermal conduction.

We analyzed Hall and Nernst coefficients (*23*) to elucidate the physical origin of the enhancement in *zT*. The Hall coefficient R_H of $Tl_{0.02}Pb_{0.98}Te$ is nearly temperature independent up to 500 K, corresponding to a hole density of 5.3×10^{19} cm^{-3}. The room temperature hole mobility μ ($\mu = R_H/\rho$) for $Tl_{0.02}Pb_{0.98}Te$ varies from sample to sample between 50 and 80 cm^2/Vs and is a factor of 5 to 3 smaller than the mobility of single-crystal PbTe at similar carrier concentrations (*26*) but has a similar temperature dependence.

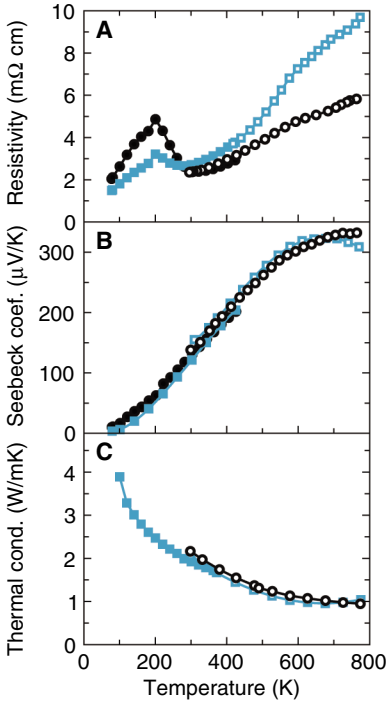

Fig. 2. Temperature dependence of the (**A**) resistivity, (**B**) Seebeck coefficient, and (**C**) thermal conductivity of a representative sample of $Tl_{0.02}Pb_{0.98}Te$ and of $Tl_{0.01}Pb_{0.99}Te$, using the same conventions as in Fig. 1. The open and closed symbols represent data taken in two different measurement systems.

As seen in Eq. 3, typically *S* depends strongly on carrier density. The solid line, known as a Pisarenko plot (*27*), shown in Fig. 3, was calculated given the known band structure and acoustic phonon scattering; almost every measurement published on *n* or *p*-type bulk PbTe falls on that line (*25*). Compared to this, *S* of Tl-PbTe at 300 K is enhanced at the same carrier concentration, as shown graphically in Fig. 3, where we show data on every Tl-PbTe sample measured in this study. All show an enhancement in *S* by a factor of between 1.7 and 3, which, in $Tl_{0.02}Pb_{0.98}Te$ samples, more than compensates for the loss in mobility in *zT*. The enhancement increases with carrier density, and indeed so does the *zT*.

We recall from Eq. 2 that *S* is a function of the energy dependence of both the density of states and the mobility. The mobility can be represented in terms of a relaxation time τ and a transport effective mass m^*: $\mu = q\tau/m^*$. The energy dependence of the relaxation time ($\tau(E) = \tau_0 E^{\Lambda}$) (*25*) is taken to be a power law, with the power, the scattering exponent Λ, determined by the dominant electron scattering mechanism. Acoustic phonon scattering in a three-dimensional solid is characterized by $\Lambda = -1/2$.

Nernst coefficient measurements (*23*) make it possible to determine the scattering exponent Λ and to decide which of the two terms in Eq. 2 dominates. We use the "method of the four coefficients" (*28*), developed to deduce μ, Λ, m^*_d and E_F from measurements of ρ, R_H, *S*, and *N*. We observe no increase in Λ over its value (−1/2) in pure PbTe (*28*) as would be expected from the "resonant scattering" (*29*) hypothesis. Furthermore, the effects of resonant scattering (*29*) would be expected to vanish with increasing temperature, because acoustic and optical phonon scattering would then become ever more dominating. This would not only contradict the results of Fig. 1 but also preclude the use of the mechanism in any high-temperature applications such as electrical power generators.

In contrast to the constant scattering exponent Λ, the method of four coefficients shows a factor of 3 increase in the effective mass (m_d^*) over that of Na-PbTe (Fig. 4) (*30*) calculated at E_F = 50 meV for a classical nonparabolic band (*25*). As seen in Eq. 3, such an increase in m_d^* will directly increase *S* by the same factor, as observed. It is also consistent with the measurements of the electronic specific heat (*22*), as expected because both the specific heat and *S* are closely related to the entropy of the electrons (*31*). The local increase in m_d^* implies a decidedly nonparabolic perturbation in the electron dispersion relations and the density of states.

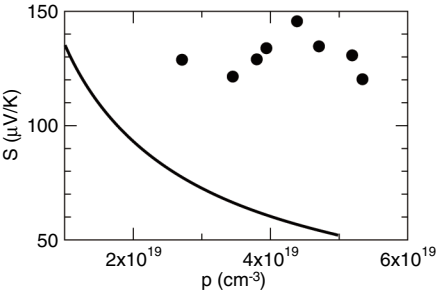

Fig. 3. Pisarenko relation of Seebeck coefficient at 300 K versus hole concentration for PbTe (solid line) compared to the results measured on every Tl-PbTe sample prepared for this study.

Because *S* and electronic heat capacity are sensitive to the change in the DOS at E_F, m_d^* derived from these quantities is actually a measure of *dn*(E)/*d*E. The latter quantity will be enhanced for E_F close to the inflection point of the *g(E)* curve (Fig. 1A), which is closer to the valence band edge than the energy at which the DOS is maximum; Indeed, there need not even be a maximum in *g(E)* for the argument to hold. The measured value of E_F at 50 meV is consistent with this description, because the inflection point is expected to be near half the energy (~30 meV in this case) at which a maximum in DOS is reported (*17*). In general, the sharper the local increase in DOS, the larger the enhancement in m_d^* and in *S*. The agreement between the measurements of the enhancement in m_d^*, specific heat, and our measured E_F for Tl-PbTe strongly supports this model as the source of enhanced *S* and *zT*.

One signature feature we observed in every Tl-PbTe sample measured is the local maximum in ρ near 200 K. It is attributed to a minimum in mobility that occurs at the same temperature at which the mass has a maximum. Thus, we suggest that the maximum in ρ, or the minimum in μ, occur at a temperature at which E_F nears an inflection point in the dispersion relation, although a much more detailed analysis involving the study of double-doped samples with variable E_Fs is necessary to reach firm conclusions.

Further improvements in *zT* should be possible by systematically searching for the optimum location of E_F compared to the shape of *g(E)*, for instance, by co-doping the samples with both Tl and another acceptor impurity such as Na. In addition to opening a new route to high-*zT* materials that is not limited by the concept of minimum κ, this approach does not rely on the

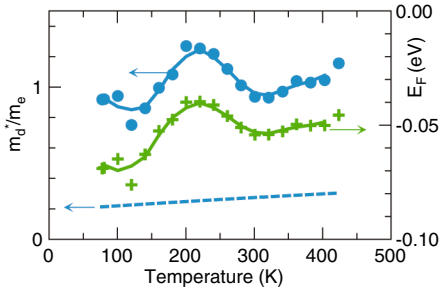

Fig. 4. Temperature dependence of the Fermi energy (green + symbols, right ordinate, the zero referring to the top of the valence band) and of the density of states effective mass (blue dots, left ordinate) of $Tl_{0.02}Pb_{0.98}Te$ compared to that of Na-PbTe (blue dashed line).

formation of nanoparticles, which are subject to grain growth or dissolution into the host material during operation. The method is independent of phonon properties, implying that improvements in zT induced by reducing the lattice κ value can work in conjunction with the mechanism described here. We anticipate that deliberately engineered impurity-induced band-structure distortions will be a generally applicable route to enhanced S and zT in all TE materials. We are optimistic about the commercial use of such PbTe-based materials because there is an extensive knowledge base among the manufacturers of thermoelectric generators about the assembly of PbTe-based devices, in particular the ability to make stable metallic contacts with low thermal and electrical resistance.

References and Notes

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35. This work was supported by the BSST Corporation, the State of Ohio Department of Development's Center for Photovoltaic Innovation of Commercialization (OSU), the Beckman Institute, the Swedish Bengt Lundqvist Minne Foundation, and Jet Propulsion Laboratory, NASA (Caltech). Patent protection related to this work is pending.

Supporting Online Material

www.sciencemag.org/cgi/content/full/321/5888/554/DC1
Materials and Methods
Fig. S1
References

28 April 2008; accepted 17 June 2008
10.1126/science.1159725

BSKs Mediate Signal Transduction from the Receptor Kinase BRI1 in *Arabidopsis*

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Brassinosteroids (BRs) bind to the extracellular domain of the receptor kinase BRI1 to activate a signal transduction cascade that regulates nuclear gene expression and plant development. Many components of the BR signaling pathway have been identified and studied in detail. However, the substrate of BRI1 kinase that transduces the signal to downstream components remains unknown. Proteomic studies of plasma membrane proteins lead to the identification of three homologous BR-signaling kinases (BSK1, BSK2, and BSK3). The BSKs are phosphorylated by BRI1 in vitro and interact with BRI1 in vivo. Genetic and transgenic studies demonstrate that the BSKs represent a small family of kinases that activate BR signaling downstream of BRI1. These results demonstrate that BSKs are the substrates of BRI1 kinase that activate downstream BR signal transduction.

Cell-surface receptor kinases activate cellular signal transduction pathways upon perception of extracellular signals, thereby mediating cellular responses to the environment and to other cells. The *Arabidopsis* genome encodes more than 400 receptor-like kinases (RLKs) (*1*). Some of these RLKs function in growth regulation and plant responses to hormonal and environmental signals. However, the molecular mechanism of RLK signaling to immediate downstream components remains poorly understood, as no RLK substrate that mediates signal transduction has been established in *Arabidopsis* (*2*). BRI1 is an RLK that functions as the major receptor for the steroid hormones brassinosteroids (BRs) (*2*). BRs bind the extracellular domain of BRI1 to activate its kinase activity, initiating a signal transduction cascade that regulates nuclear gene expression and a wide range of developmental and physiological processes (fig. S1) (*3*). Many components of the BR signaling pathway have been identified, and much detail has been revealed about how BR activates BRI1 (*4*–*8*) and how phosphorylation by downstream GSK3-like kinase BIN2 regulates the activity of the nuclear transcription factors that mediate BR-responsive gene expression (fig. S1) (*3*, *9*–*13*). However, no direct interaction has been observed between BRI1 and BIN2, and it remains unclear how BRI1 kinase at the plasma membrane transduces the signal to cytoplasmic components of the BR pathway (*14*).

To identify additional components of the BR signaling pathway, we performed quantitative proteomic studies of BR-responsive proteins using two-dimensional difference gel electrophoresis (2D DIGE). Seedlings of BR-deficient *det2-1* mutant were treated with brassinolide (BL) (the most active form of BRs) or mock solution, and proteins were labeled with Cy3 or Cy5 dyes, mixed together, and separated in the same gel by 2D gel electrophoresis (2-DE). BL-induced BAK1 phosphorylation and BZR1 dephosphorylation were detected in the plasma membrane and phosphoprotein fractions, respectively (*15*), but not in total proteins (*16*). Similar to BAK1, two additional rows of spots showed a BR-induced increase of the acidic forms and a decrease of the basic forms (Fig. 1, A and B), which is consistent with BR-induced phosphorylation. Mass spectrometry analysis of these spots identified two kinases encoded by *Arabidopsis* genes At4g35230 and At5g46570, which we named BR-signaling kinases 1 and 2 (BSK1 and BSK2) (Fig. 1B and

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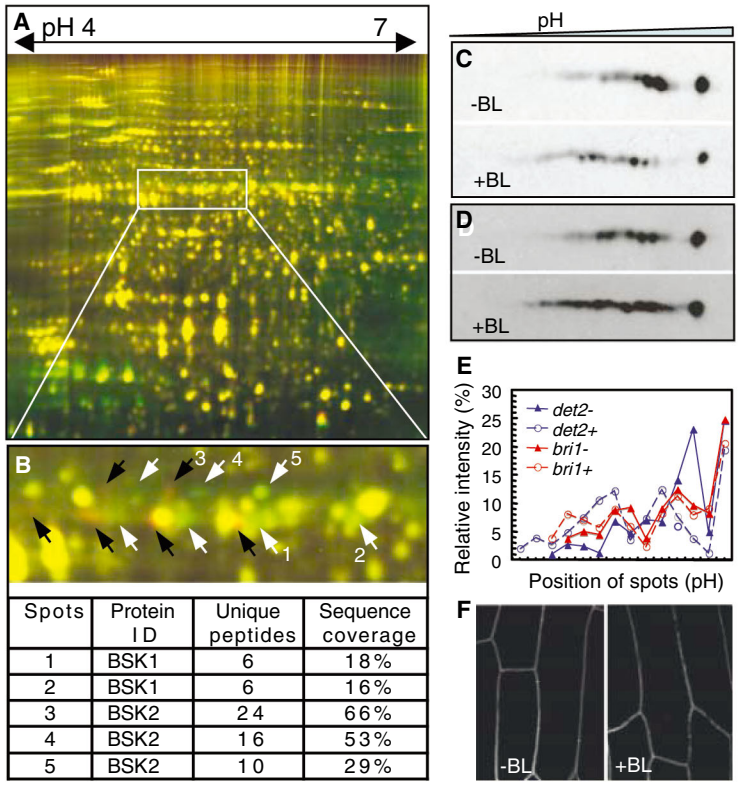

Spots	Protein ID	Unique peptides	Sequence coverage
1	BSK1	6	18%
2	BSK1	6	16%
3	BSK2	24	66%
4	BSK2	16	53%
5	BSK2	10	29%

Fig. 1. Identification of BSK1 and BSK2 as early BR-regulated plasma membrane proteins. (**A**) 2D DIGE image of plasma membrane proteins isolated from 7-day-old *det2* seedlings treated for 2 hours with either 100 nM BL (labeled with Cy5, red) or mock solution (Cy3, green). (**B**) Zoom-in view of an area in (A) showing BR-induced (black arrows, red spots) and BR-repressed (white arrows, green spots) protein spots. The table summarizes the protein identity, the number of unique peptides, and the percentage of protein sequence coverage of mass spectrometry data for the spots numbered in the upper panel. (**C** to **E**) 2D gel immunoblotting analysis of BR regulation of posttranslational modification of BSK1 in *det2* (C) and *bri1-5* (D) background. Transgenic *det2* or *bri1*-5 mutant seedlings expressing BSK1-YFP fusion protein were treated for 15 min with mock solution (-BL) or 100 nM BL (+BL). The proteins were separated by 2-DE and immunoblotted with anti-YFP antibody. (E) Quantitation of relative spot intensity along the isoelectric focusing dimension in (C) and (D). (**F**) Confocal microscopy images show localization of BSK1-YFP in hypocotyl cells of 3-day-old dark-grown transgenic *det2* seedlings before (-BL) and 2 hours after treatment with 100 nM brassinolide (+BL). Scale bar, 10 μm.

fig. S2). BSK1 and BSK2 share 60% amino acid sequence identity (fig. S3) and are members of the receptor-like cytoplasmic kinase subfamily RLCK-XII (*1*). The RLCK-XII subfamily includes 12 *Arabidopsis* proteins that each contains a kinase domain at the N-terminal side and tetratricopeptide repeat (TPR) domains at the C terminus (fig. S3) (*1*). TPR domains are known to mediate protein-protein interactions and are present in components of steroid receptor complexes in animals (*17*). BSK1 and BSK2 do not contain predicted transmembrane domains but have putative N-terminal myristylation sites (glycine 2) that could mediate their membrane localization (fig. S3).

The BR-induced shift of BSK1 from the basic to the acidic side in 2-DE gels was confirmed by immunoblotting of transgenic plants expressing a BSK1–yellow fluorescence protein (YFP) fusion protein (Fig. 1, C and E). The response was obviously weaker in the *bri1-5* mutant background (Fig. 1, D and E), suggesting that BR regulation of BSK1 is BRI1-dependent. Consistent with their identification in the plasma membrane fractions, BSK1-YFP fusion proteins showed localization on the cell surface, and the localization was not affected by BL treatment (Fig. 1F).

The plasma membrane localization and BR-induced modification of BSKs suggest that they might be substrates of BRI1 or BRI1's coreceptor kinase BAK1 (*18*, *19*). In vitro kinase assays demonstrated that BRI1, but not BAK1, phosphorylates BSK1 (Fig. 2A). Mass spectrometry analysis of BRI1-phosphorylated BSK1 identified Ser^{230} of BSK1 as a BRI1 phosphorylation site (fig. S4). This same residue is also phosphorylated in vivo (*20*). Whereas deletion of the C-terminal TPR domain has no effect on BSK1 phosphorylation by BRI1, a Ser^{230} → Ala^{230} (S230A) mutation reduced the phosphorylation by 82% (Fig. 2B), indicating that Ser^{230} is the major site for BRI1 phosphorylation.

We demonstrated in vivo interactions with BRI1 with the use of bimolecular fluorescence complementation (BiFC) and coimmunoprecipitation assays. Whereas cells coexpressing BSK1

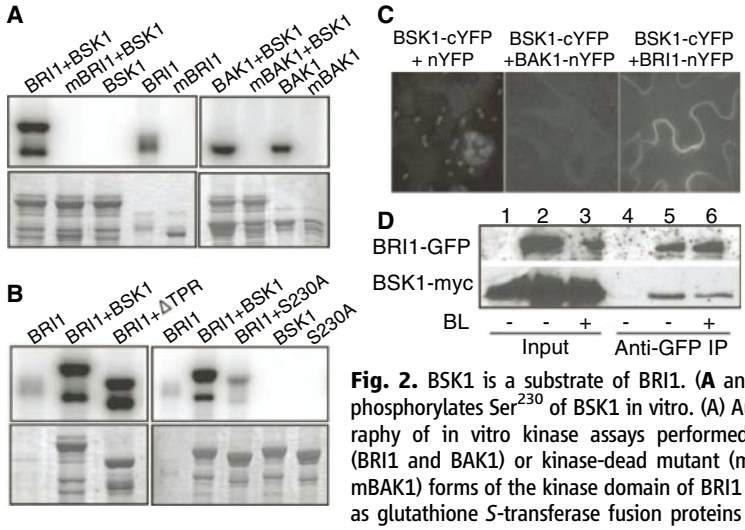

Fig. 2. BSK1 is a substrate of BRI1. (**A** and **B**) BRI1 phosphorylates Ser^{230} of BSK1 in vitro. (A) Autoradiography of in vitro kinase assays performed with WT (BRI1 and BAK1) or kinase-dead mutant (mBRI1 and mBAK1) forms of the kinase domain of BRI1 and BAK1 as glutathione *S*-transferase fusion proteins (B). In vitro kinase assays of BRI1 phosphorylation of full-length BSK1, a truncated BSK1 with deletion of the TPR domain (ΔTPR) and the S230A mutant BSK1. (**C**) BiFC assay shows BRI1 interaction with BSK1. YFP fluorescence images of *Nicotiana benthamiana* leaf epidermal cells cotransformed with the indicated constructs. (**D**) Coimmunoprecipitation of BRI1 with BSK1. *Arabidopsis* plants expressing BSK1-myc only (lanes 1 and 4) or coexpressing BSK1-myc and BRI1-GFP (lanes 2, 3, 5, and 6) were treated with 100 nM brassinolide (BL+) or mock solution (BL-) for 30 min. Microsomal proteins (lanes 1 to 3) were immunoprecipitated with anti-GFP antibodies (lanes 4 to 6), and the immunoblot was probed with anti-GFP antibodies or anti-myc antibodies.

fused to the C-terminal half of YFP (BSK1-cYFP) and the nonfusion N-terminal half of YFP (nYFP) or BAK1-nYFP fusion showed no or weak fluorescence signals (Fig. 2C), cells coexpressing BRI1-nYFP and BSK1-cYFP showed strong BiFC fluorescence at the plasma membrane (Fig. 2C). Antibodies to BSK1 (anti-BSK1) immunoprecipitated the BRI1–green fluorescent protein (GFP) protein expressed from the BRI1 promoter (fig. S5), and a BSK1-myc protein was immunoprecipitated by anti-GFP antibodies only in transgenic *Arabidopsis* plants expressing both BRI1-GFP and BSK1-myc (Fig. 2D). BR treatment reduced the amount of the coimmunoprecipitated BSK1-myc to 46% of the untreated sample (Fig. 2D), suggesting that BSK1 might be released from BRI1 upon phosphorylation. These results indicate that BSK1 is a BRI1 kinase substrate that is phosphorylated upon BR activation of BRI1.

To determine the functions of BSKs and their homologs in BR signaling, T-DNA insertion mutants were obtained for *BSK2*, *BSK3*, *BSK4*, *BSK5*, and *BSK12* genes (*21*). Of these, only the *bsk3-1* mutant showed an obvious phenotype (fig. S6). The *bsk3-1* mutant contains a T-DNA insertion in the 5′ untranscribed region and expresses a much reduced level of the *BSK3* RNA (Fig. 3, A and B). The *bsk3-1* mutant seedlings grown in the dark on regular medium or medium containing the BR biosynthetic inhibitor brassinazole (BRZ) showed shorter hypocotyl length than did wild-type (WT) seedlings (Fig. 3C). BL treatment increases hypocotyl elongation and inhibits root growth in WT plants grown in the light. Compared to the wild type, the *bsk3-1* mutant showed reduced responses to BL in hypocotyl elongation, root inhibition, and expression of the BZR1 target gene *DWF4* and the BES1 target gene *SAUR-Ac* (Fig. 3D and fig. S7). These results demonstrate that loss-of-function mutation of *bsk3* reduces BR sensitivity, indicating an essential role for BSK3 in BR signaling. Similar to BSK1, the BSK3 protein is also regulated by BR (fig. S8), is phosphorylated by BRI1 kinase in vitro (fig. S9), and interacts with BRI1 in a BR-dependent manner in vivo (fig. S10). BSK1 and BSK3 are expressed in similar tissues as is BRI1 (fig. S11). These results suggest that BSK3 and its homologs play redundant or overlapping roles in BR signaling, which could explain the weak BR-insensitive phenotypes of *bsk3-1*.

When overexpressed in the BR-insensitive *bri1-5* mutant (Fig. 4, A and B, and fig. S12) or BR-deficient *det2-1* (fig. S13) mutant backgrounds, BSK1, BSK3, and BSK5 obviously suppressed the dwarf phenotypes of the mutants. Consistent with reduced BR sensitivity of the *bsk3-1* mutant, overexpression of BSK3 is most effective in rescuing the *bri1* phenotypes. The growth phenotypes correlated with altered expression of the BZR1 target gene *DWF4* (Fig. 4C and fig. S12), indicating that overexpression of the BSKs activates downstream BR signaling. Overexpression of BSK3 partly suppressed the dwarf phenotype of the null allele *bri1-116* (Fig. 4D), but not that of the *bin2-1* mutant (Fig. 4E), indicating that BSK3 functions downstream of BRI1 but upstream of BIN2, which is consistent with BSK3 being a substrate of the BRI1 kinase.

We used quantitative proteomics to identify BSKs as previously unrecognized BR signal transduction components. This study demonstrates that sample prefractionation followed by 2D DIGE is a powerful proteomic approach for dissecting

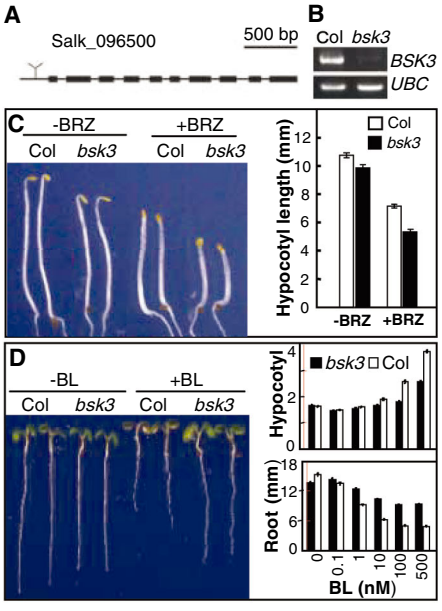

Fig. 3. The *bsk3-1* mutant has reduced BR sensitivity. (**A**) T-DNA insertion site of *bsk3-1* knockout mutant (T-DNA line SALK_096500). bp, base pair. (**B**) Reverse transcription polymerase chain reaction (RT-PCR) analysis of *BSK3* RNA expression in seedlings of WT Columbia ecotype (Col) and the *bsk3-1* mutant, with *UBC* RNA as control. (**C**) WT (Col) and *bsk3-1* seedlings grown in the dark for 4 days on a regular medium (-BRZ) or a medium containing 1 μM brassinazole (+BRZ). Average hypocotyl length of at least 25 seedlings is shown at right. Error bars indicate SE. (**D**) The *bsk3-1* mutant shows reduced sensitivity to BL. The left panel shows representative seedlings of Col or *bsk3-1* grown in the absence (-BL) or presence (+BL) of 50 nM BL for 7 days under constant light. The right panel shows hypocotyl and root lengths (average of at least 60 seedlings) of WT (Col) and *bsk3-1* seedlings grown on various concentrations of BL under continuous light. Error bars indicate SE.

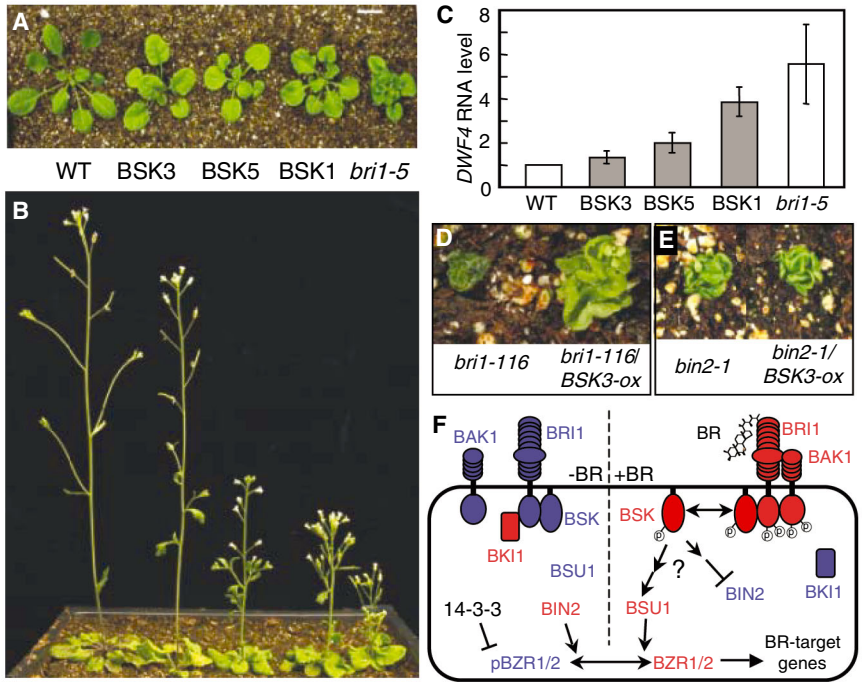

Fig. 4. BSKs function downstream of BRI1 but upstream of BIN2 in the BR signaling pathway. Phenotype of light-grown 3-week-old (**A**) or 5-week-old (**B**) WT, *bri1-5*, or transgenic *bri1-5* overexpressing BSK3, BSK5, or BSK1 as YFP fusion proteins. (**C**) Quantitative RT-PCR analysis of *DWF4* RNA expression in plants represented in (A). Error bars indicate SD. (**D**) Overexpression of BSK3 partly suppresses the *bri1-116* mutant. (**E**) Overexpression of BSK3 cannot suppress the *bin2-1* mutant. (**F**) A model of BR signal transduction. Components in the inactive and active states are shown in blue and red, respectively. In the absence of BR (-BR), BRI1 associates with BSKs in an inactive state; BIN2 phosphorylates BZR1 and BZR2 to inhibit their DNA binding activity and promote their cytoplasmic retention by the 14-3-3 proteins. BR-binding (+BR) to BRI1 induces its dimerization with BAK1 and activation of BRI1 kinase, which phosphorylates BSKs. Phosphorylated (p) BSKs dissociate from BRI1 and presumably inhibit BIN2 kinase and/or activate BSU1 phosphatase through yet unknown mechanisms, leading to dephosphorylation of BZR1 and BZR2, which regulate BR-responsive gene expression.

signaling pathways. Although only BSK1 and BSK2 were identified in the proteomic study, additional members (BSK3 and BSK5) of this family of RLCKs appear to play a similar role in BR signaling. Our results support a model for the function of BSKs in BR signaling (Fig. 4F). In the absence of BR, BSKs are associated with BRI1. Upon BR activation of BRI1, BSKs are phosphorylated and then disassociate from the receptor complex to activate downstream signaling. Such ligand-induced disassociation from a preexisting receptor complex potentially provides faster signaling than does ligand-induced recruitment of a free component into the receptor complex.

Both BSKs and BAK1 are substrates of the BRI1 kinase, but several lines of evidence indicate that they play distinct roles in BR signaling. First, BR induces BRI1-BAK1 interactions (*6*) but reduces BRI1-BSK1 and BRI1-BSK3 interactions. Second, overexpression of BSK3 suppresses the *bri1-116* null allele, whereas overexpression of BAK1 only suppresses weak alleles but not a strong allele of *bri1* nor a double mutant containing the weak *bri1-5* allele and the BR-biosynthetic mutation *det2-1* (*19*). This suggests that BSK3 functions downstream of BRI1, whereas BAK1's action on the downstream BR response requires a functional BRI1. BAK1 and its homolog BKK1 are required in additional signaling pathways, and BAK1 is also a co-receptor for the FLS2 receptor kinase (a receptor for flagelin), suggesting that BAK1 is not a specific component of the BR pathway (*22*–*25*). BAK1 most likely mediates activation of BRI1 kinase rather than signal transduction to specific downstream components in the BR signaling pathway. In contrast, the BSKs directly mediate signal transduction from BRI1 to downstream BR responses (Fig. 4F). Identification of the downstream direct targets of BSKs will be the key to fully understanding how the BR signal is transduced from the cell surface to the nuclear transcription factors.

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26. We thank W. Briggs for commenting on the manuscript. Our research was supported by grants from NSF (NSF 0724688), the U.S. Department of Energy (DE-FG02-04ER15525), and NIH (R01GM066258). S.Z. and R.W. are supported by the Chinese Scholarship Council. The University of California, San Francisco, Mass Spectrometry Facility (A. L. Burlingame, Director) is supported by the Biomedical Research Technology Program of the National Center for Research Resources and grants NIH NCRR RR01614, RR012961, and RR019934.

Supporting Online Material

www.sciencemag.org/cgi/content/full/321/5888/557/DC1
Materials and Methods
Figs. S1 to S13
References

25 February 2008; accepted 24 June 2008
10.1126/science.1156973

One-Third of Reef-Building Corals Face Elevated Extinction Risk from Climate Change and Local Impacts

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The conservation status of 845 zooxanthellate reef-building coral species was assessed by using International Union for Conservation of Nature Red List Criteria. Of the 704 species that could be assigned conservation status, 32.8% are in categories with elevated risk of extinction. Declines in abundance are associated with bleaching and diseases driven by elevated sea surface temperatures, with extinction risk further exacerbated by local-scale anthropogenic disturbances. The proportion of corals threatened with extinction has increased dramatically in recent decades and exceeds that of most terrestrial groups. The Caribbean has the largest proportion of corals in high extinction risk categories, whereas the Coral Triangle (western Pacific) has the highest proportion of species in all categories of elevated extinction risk. Our results emphasize the widespread plight of coral reefs and the urgent need to enact conservation measures.

Coral reefs harbor the highest concentration of marine biodiversity. They have high aesthetic, recreational, and resource values that have prompted close scientific scrutiny, including long-term monitoring (*1*, *2*), and face increasing threats at local and global scales. Globally, rapid buildup of carbon dioxide (and other greenhouse gases) in the atmosphere is leading to both rising sea surface temperatures (with an increased likelihood of mass coral bleaching and mortality) and acidification (*3*). Ocean acidification is reducing ocean carbonate ion concentrations and the ability of corals to build skeletons (*4*). Local threats include human disturbances such as increased coastal development, sedimentation resulting from poor land-use and watershed management, sewage discharges, nutrient loading and eutrophication from agrochemicals, coral mining, and overfishing (*1*, *2*, *5*–*9*). Local anthropogenic impacts reduce the resilience of corals to withstand global threats, resulting in a global deterioration of reef structure and ability of these ecosystems to sustain their characteristic complex ecological interactions (*1*–*3*, *5*–*9*).

In view of this ecosystem-level decline, we used International Union for Conservation of Nature (IUCN) Red List Categories and Criteria to determine the extinction risk of reef-building coral species. These criteria have been widely used and rely primarily on population size reduction and geographic range information to classify, in an objective framework, the extinction risk of a broad range of species (*10*). Categories range from Least Concern, with very little probability of extinction, to high risk, Critically Endangered (Table 1). The threatened categories (Vulnerable, Endangered, and Critically Endangered) are intended to serve as one means of setting priority measures for biodiversity conservation.

Our assessments of extinction risk cover all known zooxanthellate reef-building corals and include 845 species from the Scleractinia plus reef-building octocorals and hydrocorals (families Helioporidae, Tubiporidae, and Milleporidae). Corals have persisted for tens of millions of

years, and the many widespread species in particular are not obvious candidates for extinction. However, periods of mass coral extinctions are known from the fossil record (*11*, *12*), so conditions must have persisted that allowed populations to be reduced below sustainable levels. Up to 45% of all coral species went extinct around the Cretaceous-Tertiary boundary, with significantly more zooxanthellate than azooxanthellate extinctions (*13*). With reports of current widespread reef destruction (*2*) and unprecedented population declines in particular species (*14*, *15*), we used IUCN Red List Criteria to investigate whether present conditions have placed corals at elevated extinction risk.

Nearly all extinction risk assessments were made with the IUCN criterion that uses measures of population reduction over time (*10*). Most reef-building corals do not have sufficient long-term species-specific monitoring data to calculate actual population trends; consequently we used widely cited and independently corroborated estimates of reef area lost (*2*, *10*) as surrogates for population reduction. These estimates suffer from lack of standardized quantitative methodology, and so we interpreted them conservatively and weighted declines both regionally and by species-specific life history traits, including susceptibility to the threats causing reef area declines (*10*). Therefore, rates of population decline for each species have their basis in the rate of habitat loss within its range adjusted by an assessment of the species-specific response to habitat loss (so more-resilient species have slower rates of decline) (*10*).

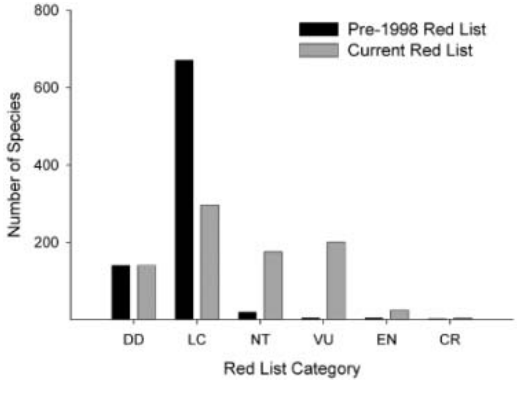
Of the 845 reef-building coral species, 141 had insufficient data to complete a Red List assessment (Table 1) and were excluded from subsequent calculations. Of the remaining 704 species, 231 are listed in the threatened categories, whereas 407 are in threatened and Near Threatened categories combined (Table 1). Species in the families Euphylliidae, Dendrophylliidae, and Acroporidae are particularly at risk, with more than or close to 50% of species in a threatened category; the figures are around 40% for Meandrinidae and Oculinidae. *Heliopora coerulea*, the sole extant member of the ancient family Helioporidae, is rated as Vulnerable. The only species that do not fall within threatened categories are those that inhabit deeper, lower reef slopes and those not solely dependent on reef habitats (i.e., inter-reefal species). The Caryophyllidae, Astrocoeniidae, Merulinidae, and Fungiidae have the lowest proportions of threatened species.

In terms of species-specific vulnerability to impacts, about 40% of the 704 species are primarily reef-restricted, shallow water corals (<20 m depth) (*10*) that are susceptible to general anthropogenic disturbances. The remaining 60% of species can survive on deeper reefs (>20 m depth), in marginal reef habitats, or in off-reef areas. There are 303 species highly susceptible to bleaching, although 102 of these typically grow quickly and

Table 1. Current Red List Categories for reef-building coral species by family. Percentages in threatened categories (Thr) include all non–data-deficient species listed as VU, EN, or CR, whereas Near Threatened and threatened (NT + Thr) include all non–data-deficient species listed as NT, VU, EN, or CR.

Family	DD	LC	NT	VU	EN	CR	Total species	NT + Thr	Thr
Acroporidae	81	54	42	85	7	2	271	71.6%	49.5%
Agariciidae	3	26	5	11			45	38.1%	26.2%
Astrocoeniidae	4	9	1	1			15	18.2%	9.1%
Caryophylliidae		3					3	0.0%	0.0%
Dendrophylliidae	1	4	3	7			15	71.4%	50.0%
Euphylliidae	3		5	9			17	100.0%	64.3%
Faviidae	5	43	57	22	3		130	65.6%	20.0%
Fungiidae	2	32	5	5	2		46	27.3%	15.9%
Helioporidae				1			1	100.0%	100.0%
Meandrinidae	3	4		2	1		10	42.9%	42.9%
Merulinidae	1	7	3		1		12	36.4%	9.1%
Milleporidae	2	8	1	2	2	1	16	42.9%	35.7%
Mussidae	7	21	12	11	1		52	53.3%	26.7%
Oculinidae	6	3	3	4			16	70.0%	40.0%
Pectiniidae	5	12	6	5	1		29	50.0%	25.0%
Pocilloporidae	2	15	5	7	2		31	48.3%	31.0%
Poritidae	10	40	20	25	5	1	101	56.0%	34.1%
Rhizangiidae		1					1	0.0%	0.0%
Siderastreidae	6	15	6	4		1	32	42.3%	19.2%
Trachyphyliidae			1				1	100.0%	0.0%
Tubiporidae			1				1	100.0%	0.0%
Total	141	297	176	201	25	5	845		

Fig. 1. Comparison of current Red List categories for all reef-building coral species to hypothetical Red List Categories back-cast to pre-1998. CR indicates Critically Endangered; EN, Endangered; VU, Vulnerable; NT, Near Threatened; LC, Least Concern; and DD, Data Deficient.



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populations recover within a few years (*7*). About 52% of the bleaching-susceptible species (mainly in the Acroporidae) are also heavily affected by disease and predation from the crown-of-thorns seastar, *Acanthaster planci*. Acroporid corals account for a high percentage of coral cover on reefs (*11*, *12*) and for a high proportion of the threatened species (Table 1). Eighty species are considered resistant to bleaching and include mostly members of the genera *Favia* and *Porites*.

Our results indicate that the extinction risk of corals has increased dramatically over the past decade (Fig. 1). By using the values from previous reports of the Global Coral Reef Monitoring Network (*16*), we determined extinction risk levels before the 1998 massive bleaching events (*10*). Before 1998, 671 of the 704 data-sufficient species would have been categorized as of Least Concern, 20 as Near Threatened, and only 13 in threatened categories. Although an estimated 6.4% of reefs recovered from the 1998 bleaching event about 5 years after it occurred, 16% were considered irreversibly destroyed after subsequent monitoring (*2*). Another study shows an increasing rate of coral cover loss in the Indo-Pacific of 1 to 2% per year since 1997 (*9*).

The proportion of threatened (not including Near Threatened) coral species exceeds that of most terrestrial animal groups apart from amphibians, particularly because of corals' apparent susceptibility to climate change (*10*). At slightly elevated sea surface temperatures, corals expel their symbionts, often resulting in colony death if the heat stress persists (*7*). Adult reef-building corals are restricted to well-lit tropical waters and are sessile, not having the option to move to cooler water. This also makes them susceptible to localized disturbances that can magnify the stress on a system already affected by warming seas.

Regionally, Caribbean reefs (Fig. 2) have been devastated by population declines of two key species, *Acropora cervicornis* (staghorn coral) and *A. palmata* (elkhorn coral) (*14*, *15*, *17*), which were recently listed as threatened under the U.S. Endangered Species Act. They were spatial dominants and primary framework builders during the Pleistocene and Holocene; their loss has had a major ecological impact (*14*, *15*). Another major Caribbean reef-builder, *Montastraea annularis*, has been listed as Endangered because of a rapid population decline over the past decade; on many

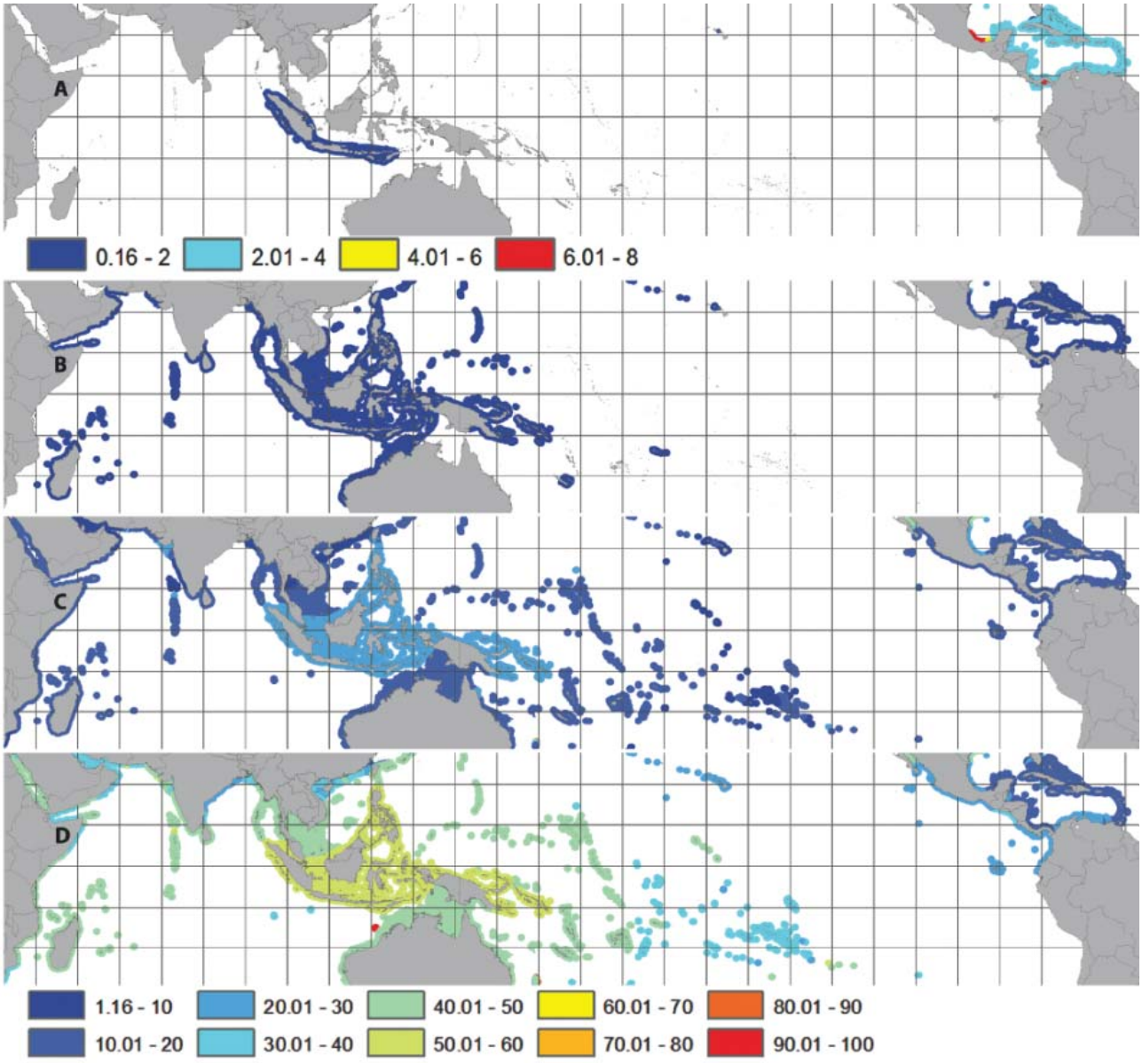

Fig. 2. (**A**) Critically Endangered species as percent of total species in area, (**B**) Critically Endangered and Endangered species as percent of total species in area, (**C**) species in all threatened categories (Critically Endangered, Endangered, and Vulnerable) as percent of total species in area, and (**D**) species in threatened and Near Threatened categories as percent of total species in area. Calculations are based on a cell size of 10 km^2.

reefs it is no longer dominant (*10*). It is the largest coral species in this region, has very slow recruitment (*18*), and is also highly susceptible to disease that can kill 500-year-old colonies within months, with recovery unlikely for decades.

In the eastern tropical Pacific, a high proportion of corals have been affected by warming events. However, subsequent monitoring has shown reefs are recovering in most areas across the region (*19*). Indian Ocean corals were the most affected by the 1998 warming event with two subsequent bleaching events in some places. Many of the shallow reefs have lost their three-dimensional rugosity, with cascading trophic and ecological effects including subsequent loss of fish populations (*20*). Other reefs are recovering their structure, but the time to complete recovery may range to decades and will be highly dependent on future climatic and local disturbance regimes.

The epicenter of marine biodiversity in the Indo-Malay-Philippine archipelago, the Coral Triangle (*11*, *21*), has the highest proportion of Vulnerable and Near Threatened coral species (Fig. 2, C and D). The chronic nature of anthropogenic disturbance in many parts of this region is compounded by the effects of climate change.

Corals in oceanic islands of the Pacific generally have the lowest proportion of threatened species (Fig. 2), and Hawaiian reefs have been spared extensive coral loss from bleaching or disease (*22*–*25*). However, Hawaii is an isolated archipelago with high levels of endemism (*23*), and several rare endemic species may prove especially vulnerable to future threats.

Our analysis indicates that the extinction risk for many corals is now much greater than it was before recent massive bleaching events. Whether corals actually go extinct this century (*12*) will depend on the continued severity of climate change, the extent of other environmental disturbances, and the ability of corals to adapt. If bleaching events become very frequent, many species may be unable to reestablish breeding populations before subsequent bleaching causes potentially irreversible declines, perhaps mimicking conditions that led to previous coral extinctions (*13*). If corals cannot adapt, the cascading effects of the functional loss of reef ecosystems will threaten the geologic structure of reefs and their coastal protection function and have huge economic effects on food security for hundreds of millions of people dependent on reef fish. Our consensus view is that the loss of reef ecosystems would lead to large-scale loss of global biodiversity.

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26. We thank T. Haas, the New Hampshire Charitable Foundation, CI, Esmée Fairbairn Foundation, and the Royal Caribbean Cruises Ocean Fund for their generous support of the IUCN Coral Red List Assessment. We also thank the following for help and support: M. Alava, J. Baillie, D. Bass, H. Reyes Bonilla, T. Brooks, H. Froyalde, P. Glynn, S. Henderson, C. Hickman, M. Hoffmann, D. Huang, R. McManus, M. Palomar, C. Pollock, R. Quicho, M. Smith, M. Syahrir, R. Trono, M. Vera, D. Zebrowski, Charles Darwin Foundation, CI Philippines, Darwin Initiative, First Philippine Conservation Incorporated, Gordon and Betty Moore Foundation, Walton Family Foundation, and the Zoological Society of London.

Supporting Online Material

www.sciencemag.org/cgi/content/full/1159196/DC1
Materials and Methods
Table S1

16 April 2008; accepted 24 June 2008
Published online 10 July 2008;
10.1126/science.1159196
Include this information when citing this paper.

Eco1-Dependent Cohesin Acetylation During Establishment of Sister Chromatid Cohesion

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Replicated chromosomes are held together by the chromosomal cohesin complex from the time of their synthesis in S phase onward. This requires the replication fork–associated acetyl transferase Eco1, but Eco1's mechanism of action is not known. We identified spontaneous suppressors of the thermosensitive *eco1-1* allele in budding yeast. An acetylation-mimicking mutation of a conserved lysine in cohesin's Smc3 subunit makes Eco1 dispensable for cell growth, and we show that Smc3 is acetylated in an Eco1-dependent manner during DNA replication to promote sister chromatid cohesion. A second set of *eco1-1* suppressors inactivate the budding yeast ortholog of the cohesin destabilizer Wapl. Our results indicate that Eco1 modifies cohesin to stabilize sister chromatid cohesion in parallel with a cohesion establishment reaction that is in principle Eco1-independent.

The cohesin complex provides sister chromatid cohesion from the time of DNA replication onward until mitosis (M) (*1*, *2*). A number of cohesion establishment factors that do not themselves form part of the cohesive structure that links sister chromatids (*3*–*9*) ensure that cohesin engages in productive linkages between sister chromatids during the synthesis phase (S phase), the period of DNA replication in the cell cycle. Of these factors, Eco1(Ctf7) is the only known essential protein. In its absence, cohesin associates with chromosomes before, during, and after S phase apparently normally (*3*, *10*), yet cohesion between sister chromatids is not established. Eco1 is a replication fork–associated acetyl transferase (*10*–*12*), suggesting a mechanistic link between replication-fork progression and cohesion establishment. How Eco1 promotes sister chromatid cohesion, and the role of its acetyl transferase activity in this process, have remained unclear.

When streaking *eco1-1* thermosensitive budding yeast cells (*3*) at their restrictive temperature, we noticed among the dying cells the outgrowth of colonies that had gained resistance to Eco1 inactivation (Fig. 1A) (*13*). Backcrossing of 20 such colonies revealed that spontaneous mutations in three complementation groups, ***a*** to ***c***, outside the *ECO1* locus, conferred thermoresistant growth. We

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tested whether the suppressor mutations restored sister chromatid cohesion in *eco1-1* cells by analyzing the green fluorescent protein (GFP)–marked *URA3* locus in metaphase-arrested cells (Fig. 1B). Mutations in all three complementation groups markedly reduced the cohesion defect in the *eco1-1* background, suggesting that they compensate for loss of Eco1 function in sister chromatid cohesion.

We identified the *eco1-1* suppressor mutations from the hybridization pattern of genomic DNA on oligonucleotide tiling arrays by means of the SNPscanner algorithm (*14*). Three members of the largest complementation group, ***b1*** to ***b3***, showed hybridization differences, as compared with the *eco1-1* parental strain, within the *RAD61* gene (Fig. 2A). Two of these strains contained nonsense mutations at the predicted positions, leading to premature termination of the open reading frame. Cells lacking Rad61 are radiation sensitive and show a weak defect in sister chromatid cohesion (*15*, *16*). Rad61 is a homolog of human and fission yeast Wapl, a protein that destabilizes cohesin on interphase chromosomes (*17*–*19*). Similar to its human counterpart, Rad61 physically associates with cohesin. It colocalizes with cohesin along chromosomes, and its absence leads to increased cohesin association with chromosomes (fig. S1). This suggests that Rad61 is the budding yeast ortholog of Wapl, and we propose that *RAD61* may also be called *WPL1*.

WPL1 is a nonessential gene, and the early stop codon in suppressor ***b1*** suggested that loss of Wpl1 function may rescue *eco1-1* cells. Consistently, deletion of *WPL1* noticeably reduced the cohesion defect in *eco1-1* cells (Fig. 2B), in agreement with observations in human cells (*18*). We next asked whether *WPL1* deletion allowed survival of cells entirely lacking Eco1. We analyzed haploid progeny after sporulation of a heterozygous diploid *eco1Δ/ECO1 wpl1Δ/WPL1* strain. As expected, *eco1Δ* spores were inviable, but *eco1Δ wpl1Δ* double-mutant spores were frequently recovered (Fig. 2C). Thus, Eco1's essential function for cell survival becomes dispensable in the absence of Wpl1. Sister chromatid cohesion in *eco1Δ wpl1Δ* double-mutant cells was compromised when compared with wild-type or *wpl1Δ* single mutants, but improved compared with *eco1-1* cells at the restrictive temperature (Fig. 2B). This demonstrates that cohesion between sister chromatids is established without Eco1 when Wpl1 is absent, although less efficiently than in Eco1's presence.

Eco1 not only promotes cohesion establishment during S phase, but also during repair of DNA double-strand breaks during G_2 (*20*, *21*). These findings open the possibility that Eco1 fulfills its truly essential role in response to DNA damage. We therefore tested the sensitivity to radiation-induced DNA breaks of *eco1-1*, *wpl1Δ*, and *eco1-1 wpl1Δ* cells. In response to 150 grays of γ-irradiation, growth of *wpl1Δ* cells was only marginally affected, consistent with its sensitivity only to high doses of radiation (Fig. 2D) (*15*). *eco1-1* cells showed marked sensitivity to this level of exposure, which was almost entirely rescued by deletion of *WPL1*. Assuming that the radiation sensitivity of *eco1-1* cells stems at least in part from Eco1's postreplicative role in double-strand break repair, this result suggests that Eco1 becomes dispensable for DNA repair in the absence of Wpl1.

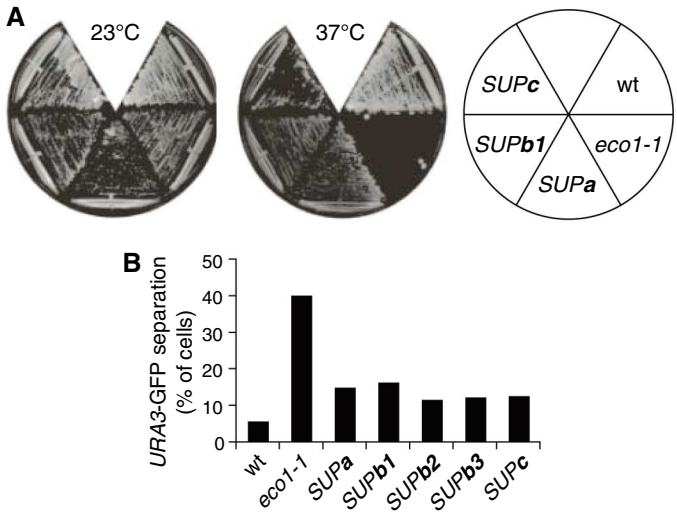


Fig. 1. Suppressors of the *eco1-1* cohesion establishment mutation. (**A**) Wild type (wt), *eco1-1*, and representatives of suppressor complementation groups ***a*** to ***c*** were streaked at both permissive (23°C) and restrictive (37°C) temperatures for the *eco1-1* allele. Note the outgrowth of two new suppressor colonies from the patch of *eco1-1* cells at 37°C shown. (**B**) Rescue of the *eco1-1* cohesion defect. wt, *eco1-1*, and the suppressor strains, including three members of complementation group ***b***, were synchronized by hydroxyurea block and released at 37°C into nocodazole-imposed metaphase arrest. Premature separation of the *URA3* locus on chromosome 5, marked with GFP, was analyzed.

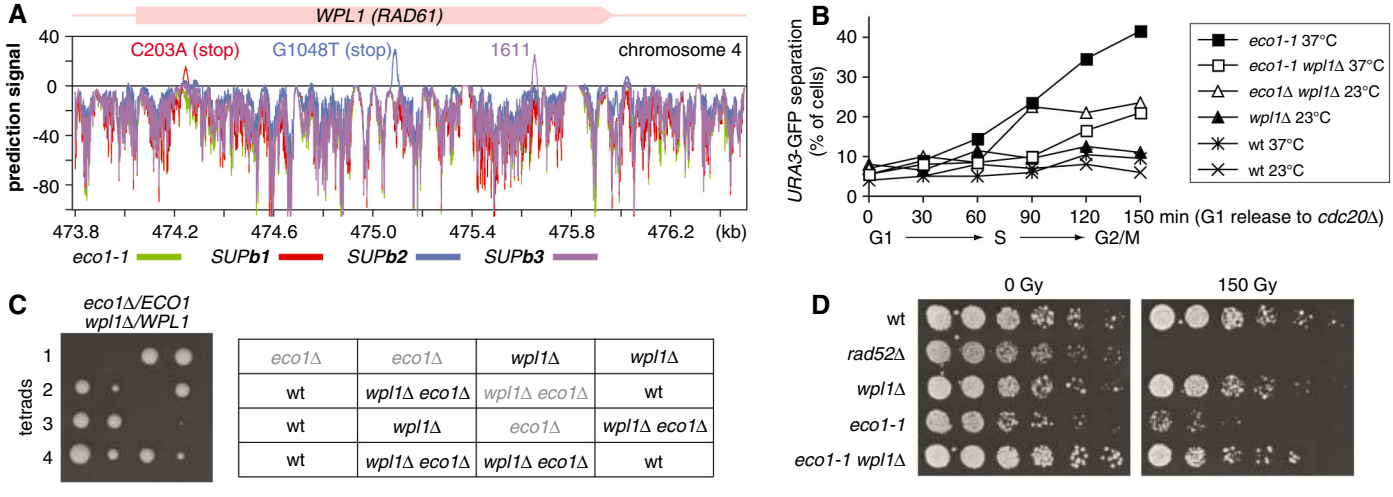


Fig. 2. Deletion of *WPL1* makes *ECO1* dispensable for sister chromatid cohesion and DNA repair. (**A**) The SNPscanner output files comparing genomic DNA from the *eco1-1* parent and three members of complementation group ***b*** are shown. (**B**) Strains of the indicated genotypes were synchronized in G_1 by α-factor block and released into metaphase arrest imposed by the depletion of the anaphase-promoting complex activator Cdc20. Cell-cycle progression, monitored by flow cytometry of DNA content, and sister chromatid cohesion at the GFP-marked *URA3* locus are indicated. (**C**) An *eco1Δ/ECO1 wpl1Δ/WPL1* heterozygous diploid was sporulated, and the genotype of the viable spores in each tetrad was analyzed. Inferred genotypes of inviable spores are in gray. (**D**) Fourfold serial dilutions of the indicated strains were spotted onto yeast extract, peptone, and dextrose agar; exposed to ^{137}Cs radiation; and incubated at 23°C. A *rad52Δ* strain served as a radiation-sensitive control.

The single mutation obtained in *eco1-1* suppressor complementation group ***a*** mapped to the gene encoding the cohesin subunit Smc3 (Fig. 3A). Sequencing revealed a single nucleotide change, resulting in a lysine-to-asparagine alteration at amino acid 113 (K113N). When reintroduced into the genome, like *WPL1* deletion, the *SMC3K113N* mutation allowed cell growth in the absence of Eco1 (Fig. 3B), indicating an important role of lysine K113 in Eco1-dependent cohesion establishment. Smc3K113 is conserved in species from yeast to human; the residue likely emerges from a surface loop on the Smc3 adenosine triphosphatase (ATPase) head domain (fig. S2) (*22*).

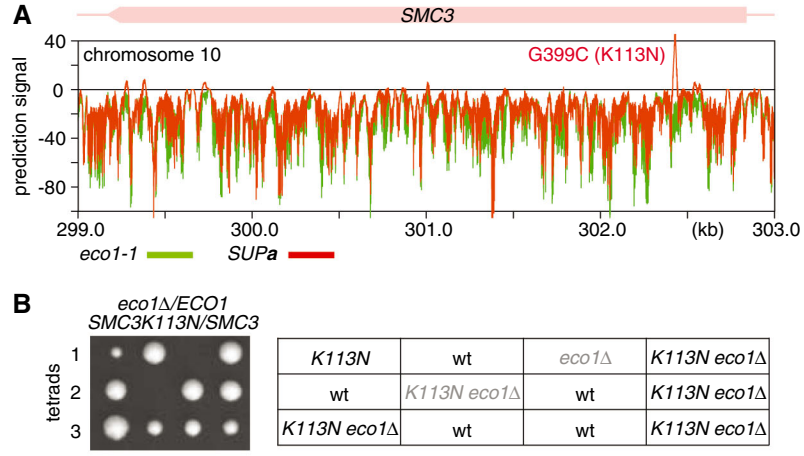
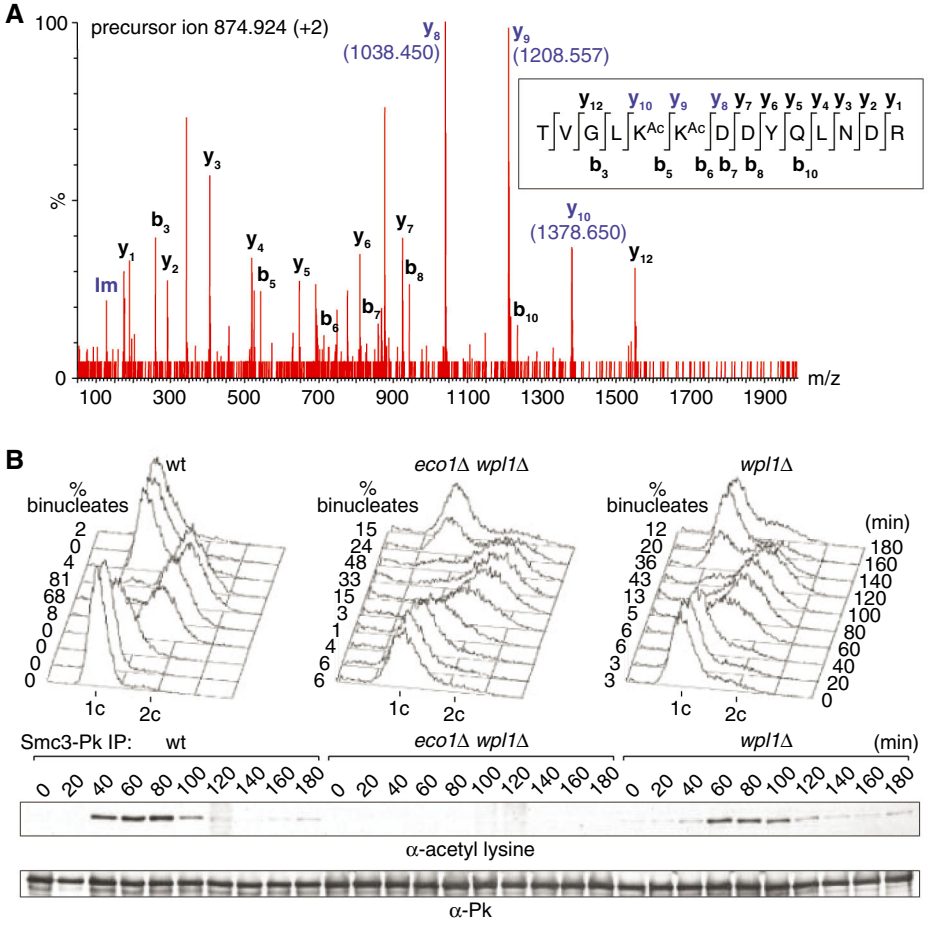


Fig. 3. Mutation of a conserved lysine in Smc3 compensates for Eco1 function. (**A**) *eco1-1* suppressor complementation group ***a*** harbors an *SMC3K113N* mutation. The SNPscanner output files comparing genomic DNA from the *eco1-1* parent and suppressor ***a*** are shown. (**B**) An *eco1Δ/ECO1 SMC3K113N/SMC3* heterozygous diploid was sporulated, and the genotype of the viable spores in each tetrad was analyzed. Inferred genotypes of inviable spores are in gray.

The biophysical properties of an asparagine side chain resemble those of acetylated lysine, suggesting that K113 may be an acetylation target. To test this, we immunopurified Smc3 from budding yeast and subjected it to mass spectrometric analysis. The fragmentation spectrum of a peptide containing K113 showed that this residue is acetylated, as is the neighboring conserved lysine K112 (Fig. 4A). To analyze when during the cell cycle acetylation occurs, we immunopurified Smc3 from a synchronous cell culture and analyzed its acetylation status by Western blotting with an α-acetyl lysine antibody (Fig. 4B). Smc3 acetylation was hardly detectable in the prereplicative phase (G_1), but increased at the time of S phase. Acetylation remained strong throughout the period between S phase and M (G_2), and diminished again as cells entered anaphase. The signal was reduced when K113 was replaced by arginine and abolished when both K112 and K113 were mutated (fig. S3). This suggests that acetylation at these two residues is recognized by the α-acetyl lysine antibody and that they are the main acetylation sites within Smc3.

To analyze whether Smc3 acetylation during S phase depends on Eco1, we compared *wpl1Δ* and *eco1Δ wpl1Δ* cells in the above time-course experiment (Fig. 4B). The timing of Smc3 acetylation and deacetylation during the cell cycle was indistinguishable between wild-type and *wpl1Δ* cells. In contrast, no Smc3 acetylation was detect-



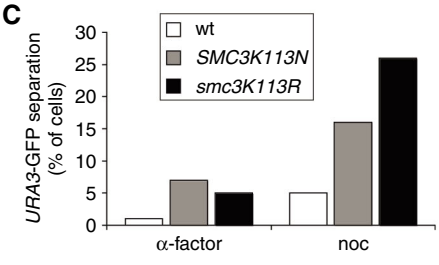


Fig. 4. Eco1-dependent Smc3 acetylation during cohesion establishment. (**A**) Mass spectrometric analysis of Smc3. The characteristic mass difference of acetyl lysines 112 and 113 in the y fragment ion series, and a diagnostic immonium ion derivative (Im), are highlighted in blue. (**B**) Smc3 was immunopurified from synchronized cultures of the indicated genotypes and its acetylation status analyzed. Cell-cycle progression was monitored by flow cytometry of DNA content, and the time of anaphase microscopically determined by the occurrence of binucleated cells. (**C**) Wild-type, *SMC3K113N*, and *smc3K113R* strains were synchronized in G_1 phase by α-factor treatment and released into nocodazole-imposed metaphase arrest. Sister chromatid cohesion at the *URA3* locus was analyzed.

able in the *eco1*Δ *wpl1*Δ strain. The Eco1 dependence is consistent with Eco1 directly acetylating Smc3. Furthermore, Wpl1 does not appear to counteract Eco1-dependent Smc3 acetylation. Rather, a destabilizing effect of Wpl1 on sister chromatid cohesion might be counteracted by Smc3 acetylation. Smc3 acetylation at the time of S phase was substantially reduced, but not abolished, when DNA replication was prevented by depletion of the replication initiation factor Cdc6 (fig. S4). This suggests that Smc3 acetylation is facilitated when Eco1 moves along chromosomes as part of the replisome (*10*, *12*) but that additional cell-cycle regulation of Eco1, or of a deacetylase that counteracts Eco1, contributes to S-phase specificity of Smc3 acetylation.

If Eco1 promotes establishment of sister chromatid cohesion by acetylating Smc3, then preventing acetylation by a lysine-to-arginine (K113R) substitution should interfere with cohesion establishment. Cells containing the *smc3K113R* mutation were viable but displayed pronounced defects in sister chromatid cohesion (Fig. 4C). Cohesion was also compromised in *SMC3K113N* cells, albeit to a lesser extent, which suggests that although asparagine 113 compensates for the requirement of Eco1, it does not support sister chromatid cohesion to the same degree as acetylated lysine. Eco1 is essential, and if its sole function in S phase is Smc3 acetylation, we would expect nonacetylatable Smc3 to cause lethality. Consistent with this expectation, cells harboring Smc3K112,113R, with both acetylated lysines replaced by arginine, were no longer viable (fig. S5). Viability was restored by deletion of *WPL1*, indicating that Smc3K112,113R is in principle proficient in sister chromatid cohesion.

In an accompanying study, Ünal *et al*. similarly describe Eco1-dependent Smc3 acetylation during S phase (*23*). These authors confirm that Smc3K112,113R supports cohesin association with budding yeast chromosomes in a manner apparently indistinguishable from wild-type Smc3, yet fails to promote sister chromatid cohesion. In contrast with our results, a single Smc3K113R mutation interfered with DNA binding and did not support cell viability. The reason for this difference is not known but could be due to different Wpl1 levels in the two strain backgrounds used for our studies. Together, our results using the Smc3K112,113R mutants suggest that Smc3 acetylation is not required for DNA binding but that Eco1-dependent acetylation of at least one of the two neighboring lysines K112 and K113 is essential to stabilize chromosome-bound cohesin at the time of cohesion establishment. Acetylation of both lysines might act in part redundantly, because an acetylation mimicking K112N mutation, like K113N, allowed growth of *eco1-1* cells at restrictive temperature, albeit not of *eco1*Δ cells (fig. S6). These results are consistent with the idea that Eco1 acts as an acetyl transferase during the establishment of sister chromatid cohesion.

The ring-shaped cohesin complex is thought to bind DNA by topological embrace (*1*, *24*). K112 and K113 emerge from the Smc3 ATPase head, where ATP hydrolysis is instrumental for cohesin's ring-opening reaction during loading onto DNA (*25*, *26*). Acetylation of these lysines could modulate cohesin's interaction with Wpl1, to prevent Wpl1 from destabilizing the cohesin ring. Alternatively, acetylation could reinforce interactions within cohesin to render it Wpl1-resistant. That the latter may be the case is suggested by our observation that Eco1 strengthens sister chromatid cohesion even in the absence of Wpl1. The position of K112 and K113 could also allow regulation of an interaction with the Smc hinge, on the opposite side of the ring, that has been implicated in ring opening (*27*, *28*). In the absence of both Eco1 and Wpl1, the fundamental mechanism for pairing sister chromatids during DNA replication remains intact. Reactions that are innate to the DNA replication process—for example, passage of the replication fork through the cohesin ring—may provide the underlying basis for sister chromatid cohesion. In wild-type cells, Eco1 modifies cohesin during DNA replication, a prerequisite for stable sister chromatid cohesion, but this can be uncoupled from S phase by *WPL1* deletion or the *SMC3K113N* mutation. We cannot exclude that the primary benefit of Wpl1-dependent cohesin regulation pertains to cohesin function outside of sister chromatid cohesion (*29*).

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30. We thank L. Kruglyak for providing the SNPscanner software; M. Ocampo-Hafalla for the cohesin chromatin immunoprecipitation data in fig. S1; E. Ünal, D. Koshland, K. Shirahige, and M. Singleton for discussions and sharing unpublished results; and members of our laboratory for comments on the manuscript. This work was supported by a European Molecular Biology Organization long-term fellowship to S.H. and by the Human Frontier Science Program. The microarray data reported in this manuscript can be obtained from Gene Expression Omnibus with accession number GSE11527.

Supporting Online Material

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Figs. S1 to S6
References

14 March 2008; accepted 24 June 2008
10.1126/science.1157774

A Molecular Determinant for the Establishment of Sister Chromatid Cohesion

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Chromosome segregation, transcriptional regulation, and repair of DNA double-strand breaks require the cohesin protein complex. Cohesin holds the replicated chromosomes (sister chromatids) together to mediate sister chromatid cohesion. The mechanism of how cohesion is established is unknown. We found that in budding yeast, the head domain of the Smc3p subunit of cohesin is acetylated by the Eco1p acetyltransferase at two evolutionarily conserved residues, promoting the chromatin-bound cohesin to tether sister chromatids. Smc3p acetylation is induced in S phase after the chromatin loading of cohesin and is suppressed in G_1 and G_2/M. Smc3 head acetylation and its cell cycle regulation provide important insights into the biology and mechanism of cohesion establishment.

Sister chromatid cohesion is required for faithful chromosome segregation and for efficient DNA double-strand break (DSB) repair and is mediated by the cohesin protein complex (Fig. 1A) (*1*–*4*). Chromatin loading per se is not sufficient for cohesin to tether sister chromatids (*3*, *5*, *6*). Eco1p (also known as Ctf7p) must act on the chromatin-bound cohesin to promote the establishment of sister chromatid cohesion both during S phase and in response to DSBs in G_2/M phase (*7*–*10*).

Eco1p possesses acetyltransferase activity (*11*–*13*). A mutant form of Eco1p, eco1p (R222G, K223G) (fig. S1), purified from bacteria has almost no detectable catalytic activity in vitro (*11*). In yeast, *eco1 (R222G, K223G)* cells are defective only in DSB-induced cohesion in G_2/M (*9*, *14*). Thus, Eco1p acetylation of cohesin seemed required for DNA damage–induced cohesion but not S-phase cohesion (*9*). However, the S-phase conclusion was challenged by two observations. First, we found that eco1p (R222G, K223G) purified from yeast extracts has auto-acetyltransferase activity in vitro (fig. S2A). Second, eco1 mutants lacking the acetyltransferase domain are inviable, a phenotype of cells defective in S-phase cohesion (fig. S2B). Together, these results suggest that the Eco1p acetyltransferase activity is required for establishing sister chromatid cohesion during S phase, and that the eco1 (R222G, K223G) protein must have sufficient acetyltransferase in vivo to carry out this function. The eco1p (R222G, K223G) protein may be unable to promote DSB-induced cohesion in G_2/M for several reasons. For instance, its acetyltransferase activity might be reduced such that it is unable to overcome an antagonizing activity (like the activity of a deacetylase), which accrues after exit from S phase. Alternatively, DSB-induced cohesion may require acetylation of DNA damage–specific targets in G_2/M that are recognized poorly by the mutant protein.

Potential acetyltransferase targets include the four cohesin subunits Smc1p, Smc3p, Mcd1p (also known as Scc1p or Rad21p), and Scc3p (Fig. 1A), as well as the cohesin-associated factor Pds5p (*7*, *15*–*19*). Mcd1p, Pds5p, and Scc3p are acetylated by Eco1p in vitro (*11*). We immunoprecipitated cohesin from extracts of asynchronous wild-type and *eco1 (R222G, K223G)* cells and observed a single acetylated band of ~150 kD, which we demonstrated to be Smc3p (Fig. 1, B and C, and fig. S2C). In *eco1-203* cells at the nonpermissive temperature, acetylated Smc3p was barely detectable (Fig. 1D and fig. S3) but was restored to wild-type levels in the presence of a plasmid-borne *ECO1* (Fig. 1D). In addition, Smc3p immunoprecipitated from yeast was acetylated in vitro by a recombinant Eco1p (Fig. 1E). Therefore, Smc3p is a bona fide substrate of the Eco1p acetyltransferase.

Liquid chromatography–mass spectrometry (LC-MS) revealed eight acetylated lysine residues in Smc3p (Fig. 1F and fig. S4). We initially focused on the conserved lysine (K) residues and mutated them either as a pair (K112, K113) or individually

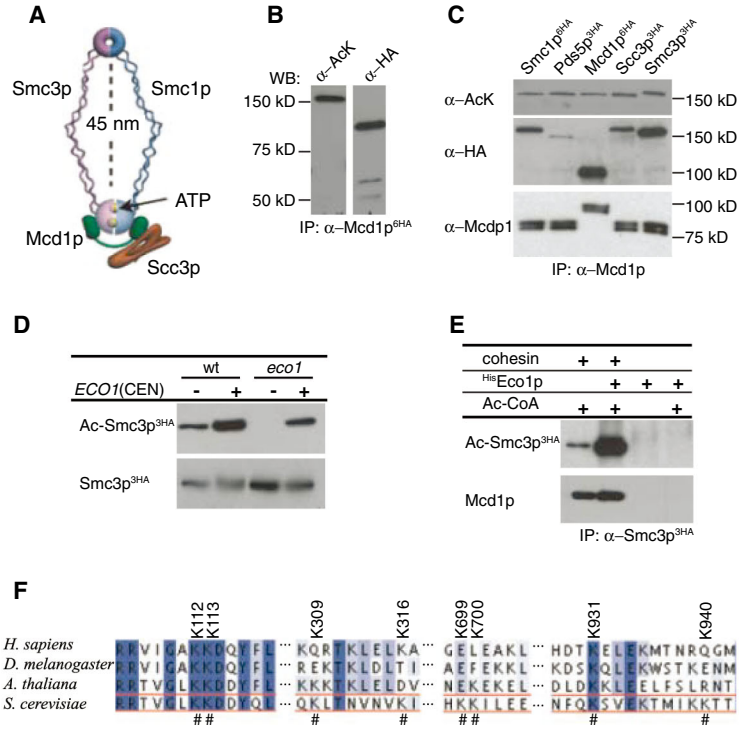

Fig. 1. Eco1p acetylates the Smc3p subunit of the cohesin complex. See (*27*) for experimental details and strains used for each experiment. (**A**) Cohesin architecture. (**B**) An acetylated protein coimmunoprecipitates with Mcd1p. WB, Western blot; IP, immunoprecipitation; HA, hemagglutinin. (**C**) Identification of the acetylated protein as Smc3p. α-AcK, antibody to acetylated lysine. (**D**) Smc3p acetylation in *eco1-203.* Ac-Smc3, acetylated Smc3p; CEN, centromeric plasmid. (**E**) Recombinant Eco1 acetylates Smc3p in vitro. Ac-CoA, acetyl–coenzyme A. (**F**) Alignment of the Smc3 orthologs using ClustalW. The amino acid numbers above the alignment correspond to the budding yeast Smc3p; # indicates the acetylated lysine residues. Abbreviations: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; Y, Tyr.

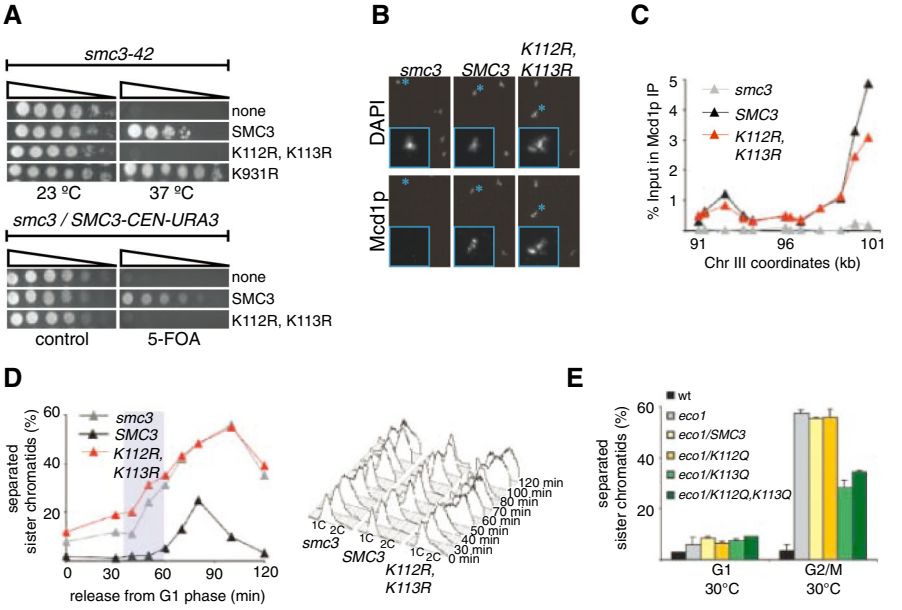

Fig. 2. Smc3p acetylation is necessary for the establishment of sister chromatid cohesion. (**A**) Spot test for growth. Triangles indicate decreasing concentration of cells in spots. 5-FOA, 5-fluoroorotic acid. (**B**) smc3p (K112R, K113R) binding to chromosomes. Each inset is a magnified view. Asterisk indicates the magnified field. DAPI, 4′,6′-diamidino-2-phenylindole. (**C**) smc3p (K112R, K113R) binding to cohesin-associated regions. (**D**) Cohesion establishment defect in *smc3 (K112R, K113R).* Violet area corresponds to S phase. (**E**) Suppression of the cohesion establishment defect in *eco1-203*. Error bars indicate SD; $n = 3$.

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(K931) to arginine (R), a structurally similar amino acid that cannot undergo acetylation. The K112R, K113R mutation, but not the K931R mutation, failed to complement *smc3* and support growth (Fig. 2A), which suggests that sister chromatid cohesion requires K112, K113 acetylation. Moreover, in the *smc3 (K112R, K113R)* mutant, as in the *eco1* mutant, cohesin associated with chromatin (Fig. 2, B and C) (*5*, *7*, *8*, *20*, *21*) but failed to establish cohesion (Fig. 2D) (*7*, *8*). This phenotypic similarity between *smc3 (K112R, K113R)* and *eco1* strongly suggests that K112, K113 acetylation by Eco1p promotes chromatin-bound cohesin to become cohesive [also reported in (*22*)]. Because both the Eco1p acetyltransferase and its functionally relevant target sites on Smc3p are conserved [(*11*–*13*) and this study], this mechanism is likely to be conserved between yeast and the other eukaryotes.

If acetylation of K112 and K113 are the only functionally important targets of Eco1p in S phase, then changing them to an acetyl-mimic glutamine (Q) (*23*) should allow cells to establish cohesion without Eco1. The presence of an ectopic copy of *smc3 (K112Q, K113Q)* restored sister chromatid cohesion in the *eco1* mutant (Fig. 2E). K113 seems to be the more critical target of the Eco1 acetyltransferase, because *smc3 (K113Q)* suppresses the cohesion defect of *eco1* to the same extent as *smc3 (K112Q, K113Q)*. However, in both *smc3 (K113Q)* and *smc3 (K112Q, K113Q)*, cohesion and growth were only partially rescued (Fig. 2E and fig. S5A). This partial suppression might reflect the presence of additional targets or the incomplete mimic of the acetylated state by glutamine substitution. Alternatively, forcing Smc3p to be acetylated constitutively could compromise sister chromatid cohesion because cohesin becomes active at the wrong time or in the wrong context.

Individual substitution mutants were generated for K112, K113, and the remainder of the Smc3 acetylation sites to assess their contribution to cohesion establishment. Only the K113R mutation failed to support growth and establish cohesion (fig. S5, B and C, and fig. S6A). Surprisingly, unlike cohesin in the *eco1* or *smc3 (K112R, K113R)* mutants, cohesin in the *smc3 (K113R)* mutant failed to associate stably with chromosomes (fig. S6, B and C). One explanation for this instability is that K113R continues to be acetylated at K112. Indeed, the monoacetylated K112 was recovered from wild-type cells (fig. S4). This monoacetylated form may normally be generated from the diacetylated K112, K113 as part of the Wapl-dependent mechanism for dissociating cohesin from chromatin in interphase and prophase (*24*–*26*). By mutating K113R, the monoacetylated K112 would be generated inappropriately, causing precocious cohesin removal. Consistent with this, deletion of RAD61, the budding yeast homolog of WAPL, restores viability and (by inference) cohesion to the K113R mutant (fig. S7). Changes in expression or Rad61p in different genetic backgrounds may explain the phenotypic difference between the K113R mutants in this and the accompanying study (*22*).

Modeling indicates that K112 and K113 are near the adenosine triphosphate (ATP)–binding pocket (fig. S8) (*6*) such that Eco1p-mediated acetylation could modulate Smc3p adenosine triphosphatase activity (*28*). This could stabilize the cohesin ring once it has embraced the sister chromatids, or could induce a conformational change to stimulate interactions within or between cohesin complex(es) (*29*). Alternatively, Smc3p acetylation could promote the dissociation of a negative cohesin regulator such as Wapl (*25*, *26*). The modification-defective and modification-mimic alleles of Smc3 provide powerful tools to test these models.

The Smc3 mutations also allowed us to identify the acetylated residue recognized by the Calbiochem antibody as K112 (fig. S9). Using this antibody, we began to assess the temporal and spatial regulation of Smc3 acetylation. Cells were sampled for K112 acetylation at regular intervals after release from arrest in G_1 or S (Fig. 3, A and B). Acetylated K112 is undetectable in G_1, accumulates during S phase, is relatively constant until G_2/M, and then diminishes when Mcd1p is degraded at the onset of anaphase to dissolve cohesion. Analysis of its spatial regulation shows that K112 acetylation also requires the Scc2p/Scc4p (*30*)–dependent loading of cohesin on chromatin (Fig. 3C). This requirement for loading might have explained the low K112 acetylation in G_1, because cohesin normally does not load at this time in budding yeast. However, even when we induced the premature loading of cohesin onto chromosomes in G_1 (Fig. 3D), K112 acetylation still remained greatly reduced relative to S-phase cells (Fig. 3E). Thus, Eco1p-dependent acetylation of Smc3p, minimally at K112 and likely at K113, occurs only upon entry into S phase and after chromatin loading of cohesin.

This regulation of Smc3 acetylation may serve several biological functions. It may ensure robust cohesion by ensuring that cohesin becomes cohesive only after binding chromatin and only in the presence of an emerging sister chromatid. In addition, it may allow cells to mark a subset of cohesins to generate two functional cohesin pools. Indeed, evidence for two pools of cohesin with different chromatin-binding properties has been found in mammalian cells (*31*). We suggest that cohesins with acetylated Smc3p may be stably bound to chromosomes and locked in their cohesive state, thereby maintaining cohesion for chromosome segregation. Cohesins lacking Smc3p acetylation may be an uncommitted reservoir that can be targeted to de novo regions of the genome to respond to dynamic processes like transcription or DNA repair.

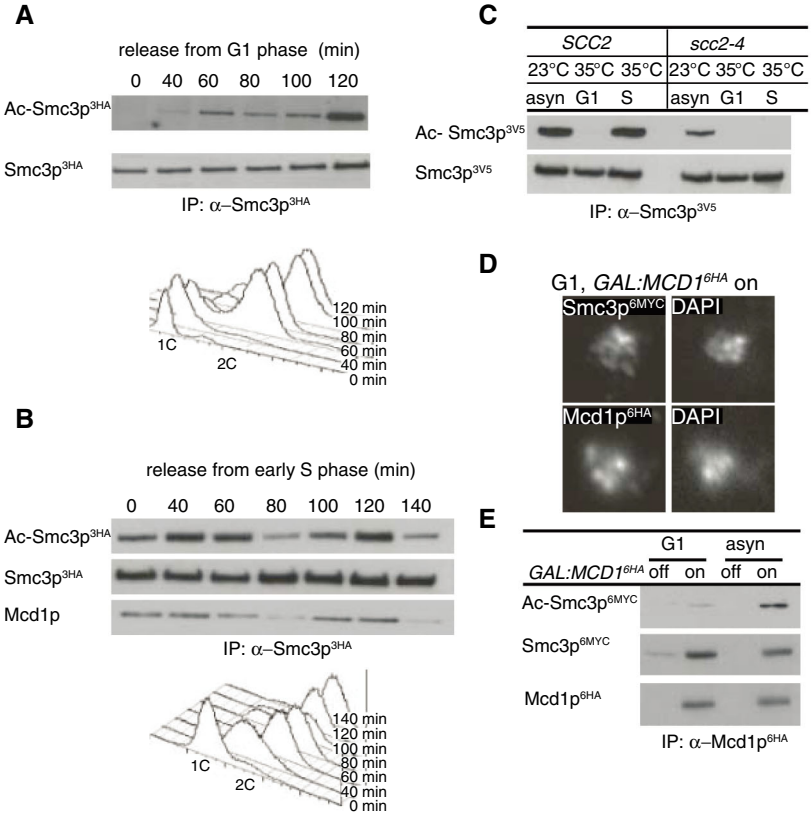

Fig. 3. Smc3p acetylation is cell cycle–regulated. (**A** and **B**) K112 acetylation during and after G_1 (A) or S phase (B). (**C**) K112 acetylation in *scc2*; asyn, asynchronous. (**D**) Induction of cohesin loading during G_1. (**E**) K112 acetylation status in G_1-loaded cohesin.

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32. We thank F. Uhlmann for communicating results before publication, M. Hoang and D. Calahan for comments on the manuscript, E. Cammon and M. Hadjithomas for technical support, and R. Sternglanz for advice and helpful discussions. We apologize to our colleagues whose work was not cited because of space limitations. D.K. is supported by the Howard Hughes Medical Institutes.

Supporting Online Material

www.sciencemag.org/cgi/content/full/321/5888/566/DC1
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Table S1
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17 March 2008; accepted 18 June 2008
10.1126/science.1157880

ERdj5 Is Required as a Disulfide Reductase for Degradation of Misfolded Proteins in the ER

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Membrane and secretory proteins cotranslationally enter and are folded in the endoplasmic reticulum (ER). Misfolded or unassembled proteins are discarded by a process known as ER-associated degradation (ERAD), which involves their retrotranslocation into the cytosol. ERAD substrates frequently contain disulfide bonds that must be cleaved before their retrotranslocation. Here, we found that an ER-resident protein ERdj5 had a reductase activity, cleaved the disulfide bonds of misfolded proteins, and accelerated ERAD through its physical and functional associations with EDEM (ER degradation–enhancing α-mannosidase–like protein) and an ER-resident chaperone BiP. Thus, ERdj5 is a member of a supramolecular ERAD complex that recognizes and unfolds misfolded proteins for their efficient retrotranslocation.

In eukaryotic cells, secretory and membrane proteins are cotranslationally translocated into the endoplasmic reticulum (ER), acquire *N*-glycans and disulfide bonds, and become folded with the help of ER-resident molecular chaperones. Correctly folded proteins exit the ER and traffic through the Golgi to their final destinations. However, if these proteins fail to acquire their correct conformation, they are recognized by ER "quality-control" mechanisms (*1*). Terminally misfolded proteins are retrotranslocated from the ER into the cytosol for degradation via the ubiquitin-proteasome system, a process known as ER-associated degradation (ERAD) (*1*).

The enzymatic modification of the *N*-linked $Glc_3Man_9GlcNAc_2$ oligosaccharide of glycoproteins (i.e., glucose, mannose, and *N*-acetylglucosamine) is recognized not only by the calnexin-calreticulin cycle for productive folding (*1*), but also by ER degradation–enhancing α-mannosidase–like protein EDEM (Htm1/Mnlp1 in yeast), or Yos 9 (yeast osteosarcoma 9) for ERAD (*2*). EDEM enhances the degradation of misfolded proteins in a mannose-trimming–dependent manner (*3*). EDEM accepts $Man_8GlcNAc_2$ substrates from calnexin (*4*, *5*) and also associates with the transmembrane proteins Derlin 2 and 3 (*6*), which in turn associate with the cytosolic p97 complex. However, EDEM itself does not bind properly folded proteins, nor does it affect their secretion or degradation (*7*).

Disulfide bonds not only stabilize protein tertiary structure, but, in the ER, they also create large oligomers of misfolded proteins (*8*) that may not be accommodated by the retrotranslocation channel. Thus, the reduction of such disulfide bonds is required for the unfolding and retrotranslocation of misfolded proteins. ERAD is accelerated by treatment with reductants, such as dithiothreitol (DTT), and is inhibited by oxidants (*7*, *9*). No protein having reductase activity in the ER has been reported to be involved in ERAD.

To elucidate the precise role of EDEM in ERAD, we screened for EDEM-binding ER proteins by an ER-membrane yeast two-hybrid system (ER-MYTHS) (*10*, *11*). In this method, the ER luminal portion of the yeast Ire1p was replaced by rat EDEM as bait and tested against a library of ER proteins as prey. Association of prey and bait causes oligomerization of Ire1p and activates its cytosolic ribonuclease activity, which leads to splicing of the mRNA of the transcriptional activator *HAC1* and, in turn, results in induction of the reporter gene (*ADE2* in this study) under control of unfolded protein–responsive elements (*UPRE*).

In a screen of a focused library of ER-resident proteins, we identified ERdj5 (JPDI) (*12*, *13*) as an EDEM-binding protein (fig. S1A). The ERdj family is comprised of five ER proteins, each containing a DnaJ domain (*14*), and ERdj5 is the only member that has thioredoxin-like domains with CXXC motifs (active cysteines with various amino acid residues between them) (Fig. 1A) (*15*). The specificity of the binding of ERdj5 to EDEM was confirmed with ER-MYTHs by using ERdj5 as bait (fig. S1B), and EDEM did not bind to any other ERdj proteins, including ERdj3 and ERdj4 (fig. S1C).

We purified recombinant mouse ERdj5 from *Escherichia coli* (*E. coli*) (Fig. 1B) and determined its reductase activity using oxidized insulin as a substrate. ERdj5 catalyzed the reduction of the insulin disulfide bonds in a dose-dependent manner in the presence of reduced glutathione (GSH), although the specific activity of ERdj5 was about one-third that of recombinant human protein disulfide reductase (PDI) (Fig. 1C). This reductase activity was not displayed by ERdj5/SS mutant, in which all cysteines of the four CXXC motifs of ERdj5 are replaced by serines (Fig. 1C). In addition, ERdj5 had neither oxidase nor isomerase activity for ribonuclease A (RNase A) and lysozyme (fig. S2).

Recombinant ERdj5 was incubated with different ratios of GSH and oxidized glutathione (GSSG) in order to determine its redox potential. The redox equilibrium constant K of ERdj5 was determined from the alkylation of free cysteines with a fluorescent maleimide (Fig. 1D). The apparent equilibrium constant (K_{app}) of ERdj5 (190 mM) was ~100 times that of the redox state of the ER lumen (*16*) (0.5 to 2.3 mM) (Fig. 1E), consistent with ERdj5's having only reductase activity. Because the redox state of the ERdj5/SS mutant did not change within the measured redox ranges (Fig. 1E), we could attribute the observed K_{app} values of ERdj5 to its CXXC motifs. The K_{app} of ERdj5 is

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the most reducing equilibrium constant reported for any ER oxidoreductase to date, which suggests that ERdj5 acts as a potent disulfide reductase under the redox conditions of the ER.

Because ERdj5 could act as a disulfide reductase and bind to EDEM, we examined the effect of ERdj5 on ERAD in vivo using two representative ERAD substrates, the null Hong Kong (NHK) variant of human α1-antitrypsin and the J chain of mouse immunoglobulin M (*8*). NHK contains one cysteine, but it aberrantly forms a disulfide-linked dimer that can be reduced to a monomer by DTT treatment, which accelerates its ERAD (*7*). Transfection of ERdj5 decreased the formation of NHK dimers after chase periods (Fig. 2, A and C). NHK was degraded much faster in ERdj5-transfected cells than in mock-transfected cells (Fig. 2, A and B). In contrast, transfection of the ERdj5/SS mutant did not inhibit dimer formation or promote the ERAD of NHK (Fig. 2, A and B, and fig. S3). Similarly, transfection with the mutants ERdj5/AA, wherein the cysteines of the four CXXC are replaced by alanine (AXXA), or ERdj5/CA, wherein the four CXXC are mutated to CXXA, did not affect the ERAD of NHK (fig. S4). An NHK/CS mutant in which the cysteine residue in NHK was mutated to serine did not form dimers and was degraded much more rapidly than wild-type NHK, regardless of the presence or absence of ERdj5 (Fig. 2D). ERdj5 overexpression did not induce the unfolded protein response (UPR) in HEK293 cells; nor was it able to induce UPR in HeLa cells, which are more stress-sensitive than HEK293 cells (fig. S5).

J chains have two inter- and three intramolecular disulfide bonds, and overexpression of J chains in HEK293 cells produced intermolecular disulfide-bonded high-molecular-weight (HMW) complexes that accumulated during the chase period (fig. S6). Transfection of wild-type ERdj5 into HEK293 cells suppressed the accumulation of HMW bands (fig. S6C) and slightly increased the

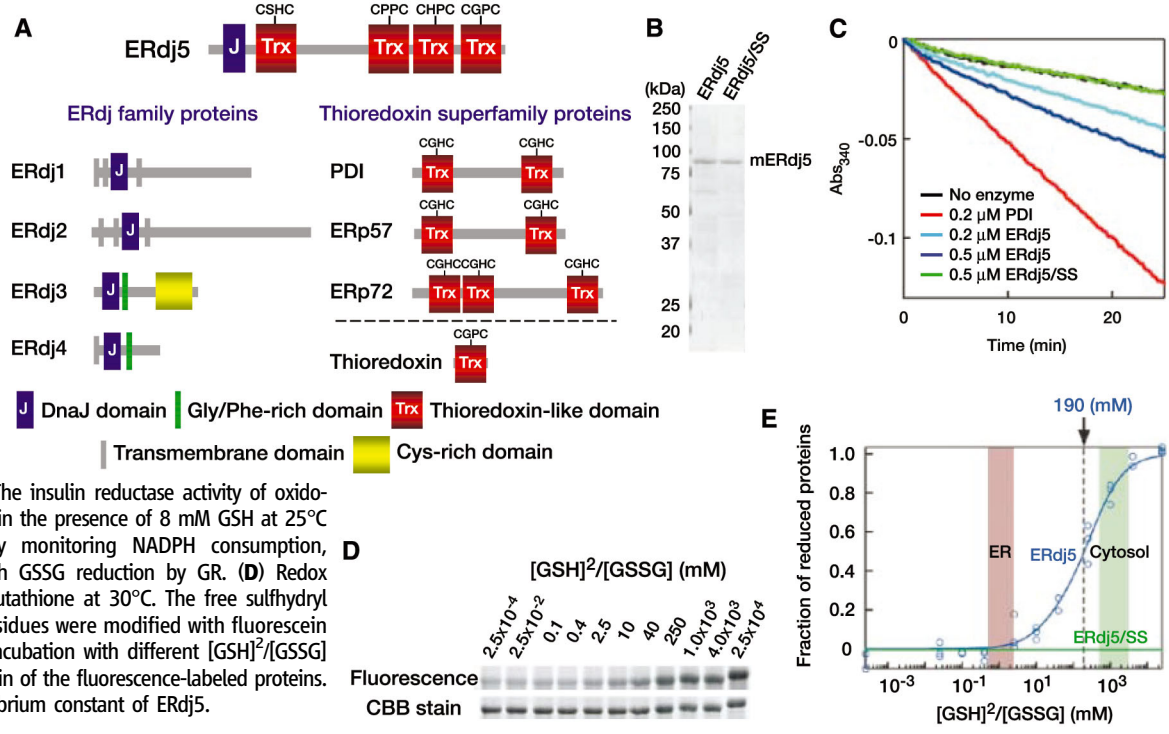

Fig. 1. Characteristics of recombinant ERdj5. (**A**) Schematic representation of the domain structures of ERdj5, ERdj family proteins, and thioredoxin superfamily proteins. (**B**) Coomassie brilliant blue (CBB) stain of purified recombinant ERdj5 and its cysteine mutant ERdj5/SS. (**C**) Insulin reductase activities of ERdj5 and PDI. The enzyme-catalyzed reduction of the insulin disulfide bonds by GSH is coupled to the reduction of GSSG to GSH by glutathione reductase (GR). The insulin reductase activity of oxidoreductases was measured in the presence of 8 mM GSH at 25°C by spectrophotometrically monitoring NADPH consumption, which is concomitant with GSSG reduction by GR. (**D**) Redox equilibrium assay with glutathione at 30°C. The free sulfhydryl groups of the cysteine residues were modified with fluorescein 5-maleimide (top) after incubation with different $[GSH]^2/[GSSG]$ ratios. (Bottom) A CBB stain of the fluorescence-labeled proteins. (**E**) Measured redox equilibrium constant of ERdj5.

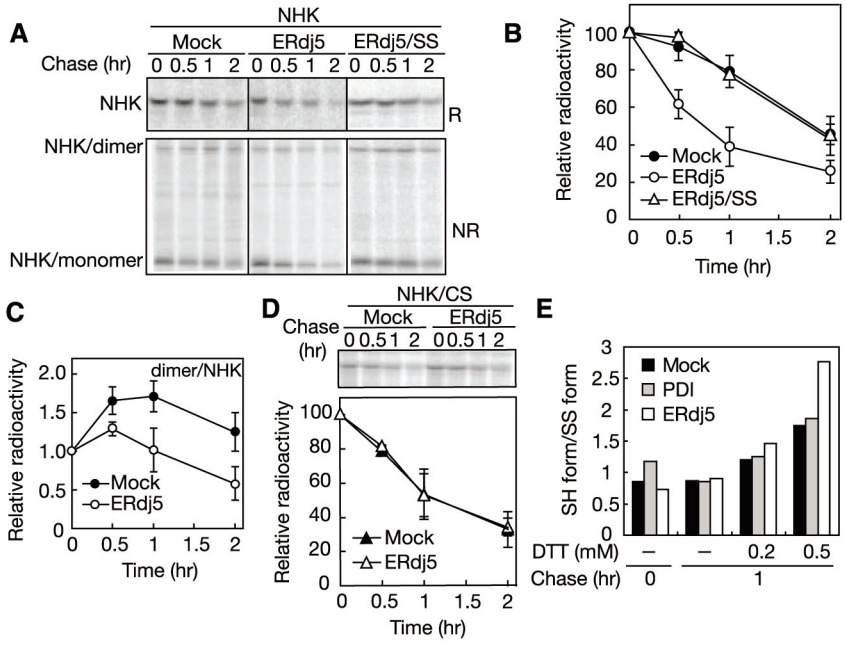

Fig. 2. Overexpression of ERdj5 accelerates ERAD for NHK. HEK293 cells were labeled for 15 min with [^{35}S]methionine-cysteine after 24 hours of transfection and chased for the periods indicated. The metabolically labeled NHK were immunoprecipitated with an α1-antitrypsin–specific antibody (**A**) and the band intensities under reducing conditions were quantified (**B**). R, reducing condition; NR, nonreducing condition. (**C**) Relative amount of the NHK dimer during the chase periods. (B) and (C) Means ± SD of three independent experiments. (**D**) Degradation of NHK cysteine-less mutant (NHK/CS) was similarly quantified. (**E**) Ratio of the free SH form of NHK to its SS form in pulse-chase experiments. HEK293 cells were labeled for 15 min with [^{35}S] after 24 hours of transfection and were chased for 1 hour in the absence or presence of 0.5 mM DTT. MG132 was added 4 hours before pulse-label to inhibit proteasome activity. Immunoprecipitation and modification of SH groups of NHK were performed as described in (*10*). The immunoprecipitants were subjected to reducing SDS–polyacrylamide gel electrophoresis, and the radioactivities of the bands of modified (SH form) and unmodified (SS form) NHK were quantified.

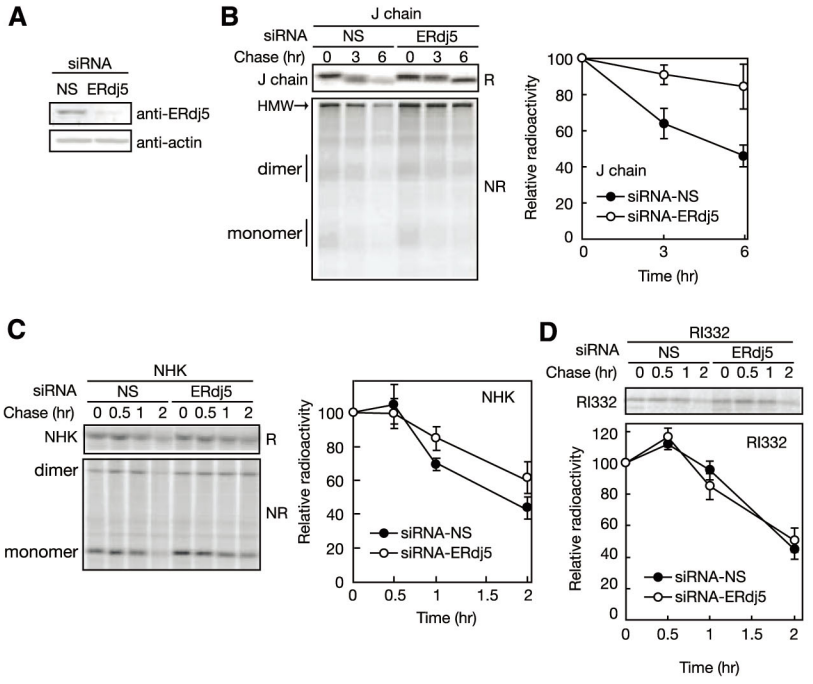

Fig. 3. Effect on ERAD of endogenous ERdj5 knockdown by the use of siRNA. (**A**) Immunoblotting of a HEK293 cell lysate with an antibody against ERdj5 shows that endogenous ERdj5 was efficiently knocked down 48 hours after siRNA transfection. (**B** and **C**) ERdj5 knockdown using siRNA in HEK293 cells slows ERAD of the J chain (B) and NHK (C). Pulse-chase experiments were performed 24 hours after transfection of ERAD substrates, which were transfected 48 hours after siRNA transfection. This degradation analysis was performed 24 hours after J chain transfection to observe the delay of degradation; this explains why the degradation profile of the J chain is different from that in fig. S6. Each graph shows the quantification of the band intensities under reducing conditions. (**D**) Knockdown of ERdj5 has no effect on the ERAD of the cysteine-less substrate, soluble ribophorin (1-332) (RI332). Results are means ±SD of three independent experiments. NS, nonspecific siRNA; R, reducing condition; NR, nonreducing condition.

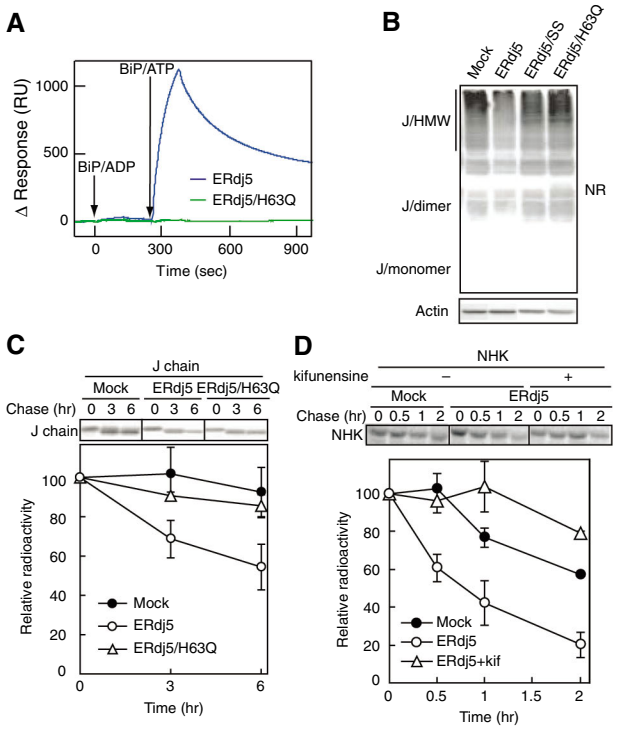

Fig. 4. Interactions of ERdj5 with EDEM and BiP have important implications for ERAD acceleration by ERdj5. (**A**) Surface plasmon resonance experiments. ERdj5 was immobilized on a CM5 chip and BiP was injected as an analyte with nucleotides. The time points of each injection are indicated by arrows. (**B**) Accumulation of a HMW complex of J chains at 48 hours after transfection was analyzed under nonreducing conditions by immunoblotting with a Myc-specific antibody. (**C**) Pulse-chase experiments of the J chain in HEK293 cells transfected with ERdj5 or the ERdj5/H63Q mutant were performed 36 hours after transfection. (**D**) ERAD promotion by ERdj5 was inhibited by adding the ER α1,2-mannosidase inhibitor kifunensine; experiments using NHK as a substrate were performed 24 hours after transfection. Transfected cells were treated with 5 μg/ml kifunensine for 4 hours before pulse-label. Results in (C) and (D) are the means ± SD of three independent experiments.

levels of monomer and dimer species (fig. S6A). ERdj5 overexpression dramatically enhanced J chain degradation compared with that in mock-transfected controls (fig. S6, A and B). The J chain mutant J/CS, in which two intermolecular disulfide-bonding cysteines were replaced with serines, did not produce HMW complexes and was degraded much more rapidly than wild-type J chains (fig S6, A and B).

If ERdj5 acts as a reductase for misfolded substrates, it should bind to NHK. The formation of a mixed disulfide complex of NHK with wild-type ERdj5 was detected in a coimmunoprecipitation analysis; such complexes were not detected with the ERdj5/AA mutant (fig. S7). We next examined the accumulation of reduced NHK in the presence of ERdj5. In the presence of the proteasomal inhibitor MG132, the ratio of the reduced and oxidized forms of NHK was not altered by ERdj5 overexpression (Fig. 2E). This may be because reoxidation of stalled substrates occurred during the chase period. However, when reducing equivalents were provided by adding low concentrations of DTT, accumulation of reduced NHK increased in the ERdj5-overexpressed cells compared with nontransfected cells (Fig. 2E). Such an increase in the reduced form of NHK was not observed when PDI was overexpressed (Fig. 2E). Thus, overexpression of ERdj5 prevents the covalent multimer formation of misfolded proteins by disulfide bond cleavage and thereby accelerates protein degradation of the ERAD substrates.

We next examined the contribution of endogenous ERdj5 to the ERAD of J chains or of NHK by transfecting HEK293 cells with an ERdj5-specific small interfering RNA (siRNA). The amount of ERdj5 in these cells was reduced to 10% of that in nonspecific siRNA-transfected controls (Fig. 3A). ER stress was not induced in cells in which expression of ERdj5 was reduced (knocked down) (fig. S5). ERdj5 knockdown induced the accumulation of HMW complexes of J chains and of NHK dimers (Fig. 3, B and C). Furthermore, the degradation of J chains and NHK was clearly inhibited in ERdj5-knockdown cells compared with nonspecific siRNA-transfected cells (Fig. 3, B and C). Finally, degradation of an ERAD substrate without cysteine, a soluble form (residues 1 to 332) of ribophorin I, was unaffected by knockdown of endogenous ERdj5 (Fig. 3D). Thus, endogenous ERdj5 is involved in the acceleration of ERAD by cleaving the disulfide bonds of misfolded proteins.

ERdj5 contains a DnaJ domain and was shown to interact with the DnaJ-binding chaperone BiP in the ER-MYTHS system (fig. S1B). We also showed that recombinant BiP binds directly to ERdj5 in an adenosine triphosphate (ATP)–dependent manner, by surface plasmon resonance by using recombinant ERdj5 immobilized on a CM5 sensor chip (Fig. 4A). Because DnaJ domain–containing proteins interact with heat shock protein 70 (Hsp70) family proteins through their His-Pro-Asp (HPD) motifs, we next exam-

ined the binding of BiP with a DnaJ-domain mutant of ERdj5 in which histidine 63 in the HPD motif is replaced with glutamine (ERdj5/H63Q) (*13*). ERdj5/H63Q did not bind to BiP, even in the presence of ATP (Fig. 4A). Thus, ERdj5 interacts through its DnaJ domain with BiP.

We examined the in vivo effect of the ERdj5/H63Q mutant on HMW complex formation and on the ERAD of J chains. Accumulation of the J chain HMW complex was considerably decreased 48 hours after transfection with wild-type ERdj5 compared with amounts after mock transfection (Fig. 4B). In contrast, neither transfection with ERdj5/H63Q nor with the ERdj5/SS mutant repressed the accumulation of J chain HMW complexes. Transfection with the ERdj5/H63Q mutant did not accelerate J-chain degradation (Fig. 4C and fig. S3) even though this mutant retains the disulfide reductase activity (fig. S2). Thus, both the reductase activity of ERdj5, which is conferred by its CXXC motifs, and the association of ERdj5 with the molecular chaperone BiP are necessary to prevent multimer formation by the misfolded proteins and also to promote efficient ERAD of such proteins.

We next examined the involvement of ERdj5 in the EDEM-mediated ERAD pathway. EDEM coimmunoprecipitated with both wild-type ERdj5 and the ERdj5/AA mutant in HEK293 cells, which suggests that the binding of EDEM with ERdj5 is CXXC-independent (fig. S8). Acceleration of ERAD by EDEM overexpression is dependent on mannose-trimming and is inhibited by kifunensine, an inhibitor of ER mannosidase I (*3*). Promotion of ERAD by ERdj5 overexpression was totally abolished in the presence of kifunensine (Fig. 4D), which suggests that ERdj5-mediated ERAD requires EDEM to function.

Thus, ERdj5, either overexpressed or endogenous, can serve as a key component for the ERAD of misfolded proteins. The following are all required for ERdj5-mediated ERAD acceleration: (i) the reductase activity of ERdj5, which is conveyed through its CXXC motif; (ii) the binding of ERdj5, through its DnaJ domain, to the ER-resident Hsp70 family chaperone BiP; and (iii) the functional interaction of ERdj5 with EDEM, a lectin-like molecule that may recognize the Man_8 *N*-glycan on misfolded proteins to be degraded. ERdj5-mediated cleavage of intermolecular disulfide bridges decreased the accumulation of covalent multimeric forms of misfolded proteins in the ER; such accumulations are expected to hinder the retrograde transport of misfolded proteins through the retrotranslocation channel. ERdj5 may prevent disulfide-linked aggregation and/or misfolding of substrates by maintaining them in reduced states and may enhance their ERAD by increasing retrotranslocation-competent misfolded proteins.

The redox potential of ERdj5 is even more reducing than the ER redox status (*16*) or the redox potential of PDI (*17*). ERdj5 has three CXPC motifs, consistent with reports that the redox potential of CXPC motifs contained in thioredoxin superfamily proteins is reducing (*18*). Its reducing redox potential indicates that ERdj5 is thermodynamically stable in an oxidized form in the ER redox environment and that ERdj5 can function as a strong disulfide reductase once it accepts electrons from electron donors. Electrons might be transported into the ER from the reducing cytosol or provided from the high ER concentration of the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) (*19*). These possibilities remain to be addressed.

Here, we have established the presence of a supramolecular functional ERAD complex, comprising EDEM, ERdj5, and BiP, which have distinct, but linked and concerted, roles (fig. S9). In this model, after the transfer of terminally misfolded proteins from calnexin to EDEM, ERdj5 bound to EDEM cleaves their disulfide bonds, which results in dissociation of the covalent multimeric substrates. At the same time, ERdj5 activates the conversion of the ATP-form of BiP to adenosine diphosphate form, resulting in dissociation of BiP from ERdj5, which, in turn, strongly binds the substrates (*20*) and holds them in a dislocation-competent state until they are transferred to the retrotranslocation channel.

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21. We thank N. Hosokawa for providing NHK and the EDEM protein plasmids. We also thank R. I. Morimoto and K. Inaba for critical discussions and advice. This work was supported by Grants-in-Aid for Creative Scientific Research (19GS0314) and Scientific Research (19058008) (to K.N.) and the Canadian Institutes of Health Research (to D.Y.T.). R.U. was supported by a fellowship from the Japan Society for the Promotion of Science.

Supporting Online Material

www.sciencemag.org/cgi/content/full/321/5888/569/DC1
Materials and Methods
Figs. S1 to S9
References

18 April 2008; accepted 12 June 2008
10.1126/science.1159293

The Crystal Structure of [Fe]-Hydrogenase Reveals the Geometry of the Active Site

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Biological formation and consumption of molecular hydrogen (H_2) are catalyzed by hydrogenases, of which three phylogenetically unrelated types are known: [NiFe]-hydrogenases, [FeFe]-hydrogenases, and [Fe]-hydrogenase. We present a crystal structure of [Fe]-hydrogenase at 1.75 angstrom resolution, showing a mononuclear iron coordinated by the sulfur of cysteine 176, two carbon monoxide (CO) molecules, and the sp^2-hybridized nitrogen of a 2-pyridinol compound with back-bonding properties similar to those of cyanide. The three-dimensional arrangement of the ligands is similar to that of thiolate, CO, and cyanide ligated to the low-spin iron in binuclear [NiFe]- and [FeFe]-hydrogenases, although the enzymes have evolved independently and the CO and cyanide ligands are not found in any other metalloenzyme. The related iron ligation pattern of hydrogenases exemplifies convergent evolution and presumably plays an essential role in H_2 activation. This finding may stimulate the ongoing synthesis of catalysts that could substitute for platinum in applications such as fuel cells.

Molecular hydrogen (H_2) is a relatively inert molecule. The dissociation energy of the H-H bond is 436 kJ mol^{-1}, and its pK_a is 35 (*1*, *2*). Despite this, H_2 is used or produced by many microorganisms in their energy metabolism. Indeed, H_2 was one of the earliest energy sources available on Earth when life evolved. H_2/H^+ interconversion reactions ($H_2 \leftrightharpoons 2H^+ + 2e^-$) are catalyzed by hydrogenases that contain nickel and/or iron as key components in their active sites; these hydrogenases are more efficient catalysts than platinum, which is used industrially to catalyze hydrogenation. H_2 is considered a major fuel in future energy technology, but the amounts of catalyst required for large-scale H_2 production and use will require alternatives to platinum, which is expensive and not abundant. One approach of current research is to learn from hydrogenases (Fig. 1), and model compounds mimicking the metal centers of hydro-

genase have recently been synthesized that can interact with protons and/or H_2 (*3*–*7*).

The most prominent hydrogenases are [NiFe]-hydrogenase (in bacteria and archaea) and [FeFe]-hydrogenase (in bacteria and eukaryotes) (*8*). The structures of their binuclear metal active sites are pictured in Fig. 1, A and B (*9*–*17*). In addition to the binuclear metal center, both types of hydrogenases harbor at least one essential [4Fe-4S] cluster. The iron in the binuclear [NiFe] center (Fig. 1A) is redox-inactive and low-spin (*18*), as probably is the iron proximal to the [4Fe-4S] cluster in the binuclear [FeFe] center (*19*) (Fig. 1B). The third type of hydrogenases, the [Fe]-hydrogenase—formerly named iron-sulfur cluster–free hydrogenase or H_2-forming methylenetetrahydromethanopterin (methylene-H_4MPT) dehydrogenase (*20*)—contains a mononuclear iron center (Fig. 1C) and is found only in some hydrogenotrophic methanogenic archaea, where it catalyzes one step involved in CO_2 reduction to methane (Fig. 2). Each subunit of the homodimeric enzyme contains one iron (which is not redox-active) and no iron-sulfur clusters.

In [Fe]-hydrogenase, the iron center is the catalytically active constituent of an iron guanylyl pyridone cofactor (FeGP cofactor), which can be extracted from the enzyme by denaturation in the presence of mercaptoethanol and used for reconstitution of the active enzyme from inactive heterologously produced apoenzyme (*21*). The FeGP cofactor is light- and temperature-sensitive (*22*), which hampers purification to homogeneity. Observed decomposition products are guanylyl pyridone (see below), two CO molecules, and one iron ion (*23*). Infrared (IR) spectroscopic analysis (*24*) revealed two CO molecules as iron ligands; x-ray absorption analysis (*25*) predicted two CO, one sulfur, and one or two N/O ligands at coordination distance to iron; Mössbauer spectroscopic data (*26*) identified the iron as low spin, either in the Fe(0) or Fe(II) oxidation state; and x-ray structure analysis of the [Fe]-hydrogenase without the FeGP cofactor (apoenzyme) established the overall architecture of the enzyme (*27*) (Fig. 3). Here, we describe the structure of the [Fe]-hydrogenase–FeGP complex (holoenzyme) at 1.75 Å resolution (Fig. 3A) (*28*). This allows a detailed three-dimensional view of the structure and binding of the intact FeGP cofactor—the site of H_2 activation—and thereby integrates previous biochemical and biophysical data into a comprehensive and consistent picture.

To elucidate the holoenzyme structure, we reconstituted the heterologously produced apoenzyme of *Methanocaldococcus jannaschii* with the labile FeGP cofactor under completely anaerobic and red-light conditions and crystallized the reconstituted enzyme. The crystal structure of [Fe]-hydrogenase contains the FeGP cofactor with a high occupancy embedded in front of the C-terminal end of the parallel β sheet of both Rossmann fold–like peripheral units and capped by an α-helical subdomain (Fig. 3). The specific amino acid residues involved in binding of the FeGP cofactor to the enzyme are shown in fig. S1. The guanosine monophosphate moiety functions to anchor the FeGP cofactor, and its binding mode essentially corresponds to the adenosine monophosphate moiety of dinucleotide binding proteins (Fig. 4). The catalytically relevant iron-center moiety is located close to the intersubunit clefts (Fig. 3) and consists of a mononuclear iron atom surrounded by a distorted square pyramidal or an octahedral ligation shell dependent on the enzymatic state (Fig. 5).

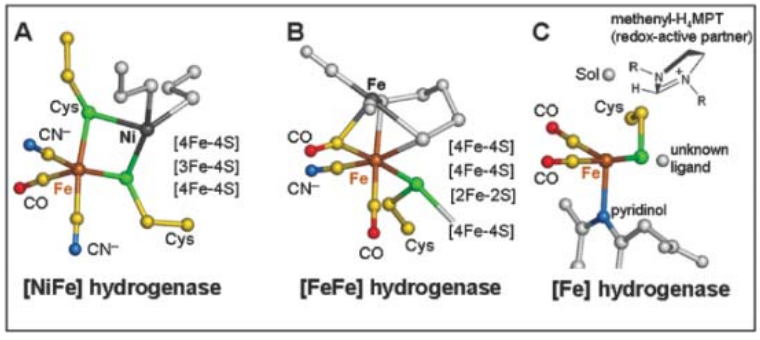
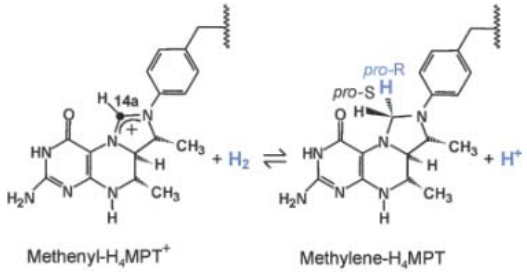


Fig. 1. Superimposed active-site structure of the three phylogenetically unrelated hydrogenases. (**A**) [NiFe]-hydrogensase from *Desulfovibrio gigas* (*9*, *12*). (**B**) [FeFe]-hydrogenase from *Clostridium pasteurianum* (*13*) and *Desulfovibrio desulfuricans* (*14*, *16*). (**C**) [Fe]-hydrogenase from *Methanocaldococcus jannaschii* (this work). In [Fe]-hydrogenase, the fifth and sixth ligation sites are marked by gray spheres. All three hydrogenase types have in common a low-spin iron (brown) ligated by thiolate(s), CO, and cyanide or pyridinol (considered as cyanide functional analog), which acts together with a redox-active partner (dark gray). The partners—Ni, the distal iron, and methenyl-H_4MPT^+ (modeled), respectively—take over the electrons or the hydride and perhaps play a role in the heterolytic cleavage of H_2.

Fig. 2. Reaction catalyzed by [Fe]-hydrogenase in methanogenic archaea. The heterolytic cleavage of H_2 by the enzyme is dependent on the presence of methenyl-H_4MPT^+, whose methenyl C14a has carbocation character and is therefore an excellent hydride acceptor. H_4MPT, tetrahydromethanopterin.



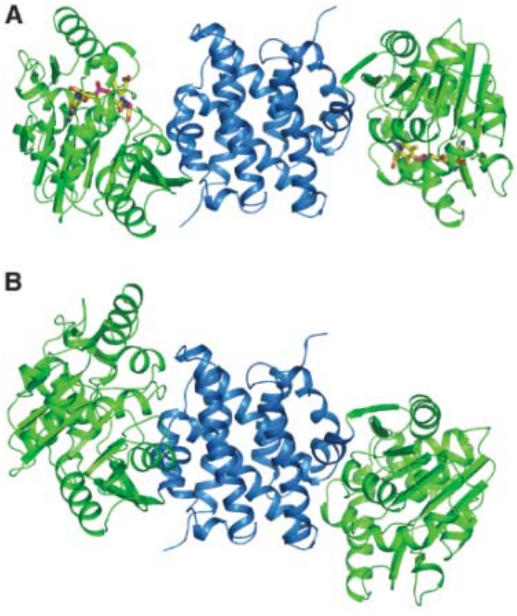


Fig. 3. Ribbon diagrams of the [Fe]-hydrogenase of *M. jannaschii*. The homodimeric enzyme is composed of three folding units. The two peripheral units (N-terminal segments) consist of a Rossmann fold–like domain (green) which can be further subdivided into a classical Rossmann fold, an extension (βαβ), and an α-helical insertion region. The unique central unit (in blue) constitutes an intertwined helix bundle formed by the C-terminal segments of both subunits (*27*). The structure of the holoenzyme (**A**) is in an open conformation and that of the apoenzyme (**B**) is in a closed conformation with respect to the cleft between the central and the peripheral units (*27*). The rotation angle between the states is 37°. The FeGP cofactor is bound to the peripheral units and is depicted as a color-coded stick model.

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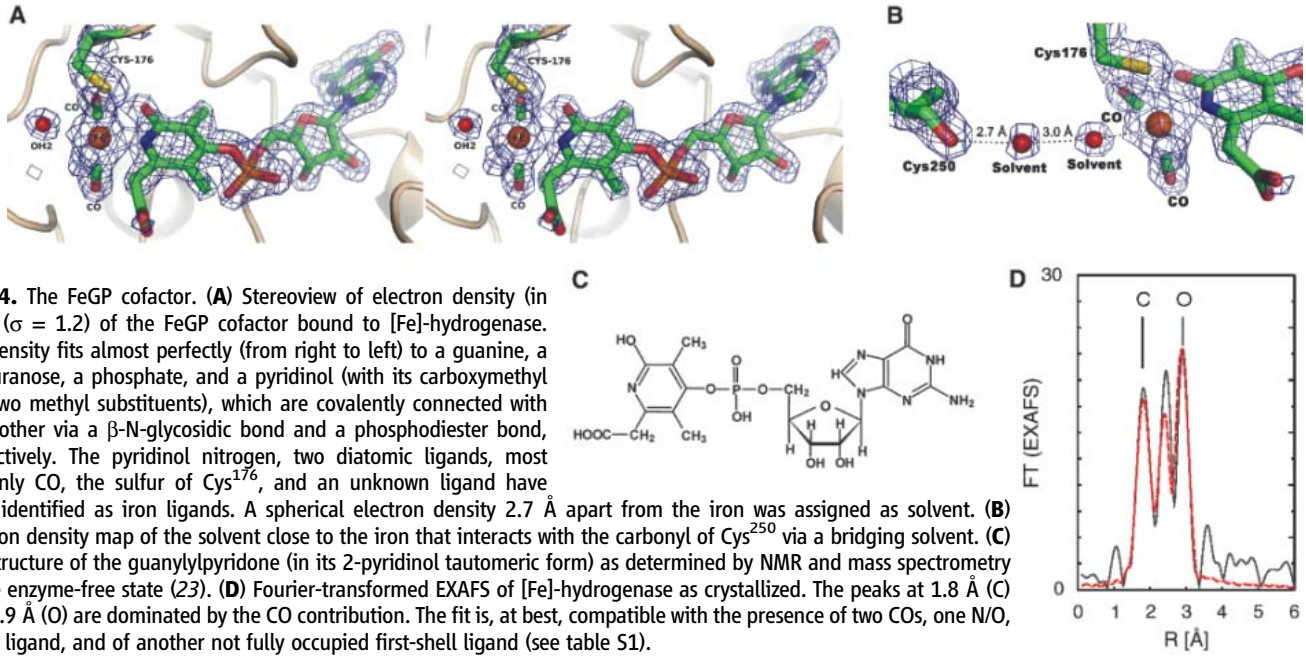

Fig. 4. The FeGP cofactor. (**A**) Stereoview of electron density (in blue) (σ = 1.2) of the FeGP cofactor bound to [Fe]-hydrogenase. The density fits almost perfectly (from right to left) to a guanine, a ribofuranose, a phosphate, and a pyridinol (with its carboxymethyl and two methyl substituents), which are covalently connected with each other via a β-N-glycosidic bond and a phosphodiester bond, respectively. The pyridinol nitrogen, two diatomic ligands, most certainly CO, the sulfur of Cys^{176}, and an unknown ligand have been identified as iron ligands. A spherical electron density 2.7 Å apart from the iron was assigned as solvent. (**B**) Electron density map of the solvent close to the iron that interacts with the carbonyl of Cys^{250} via a bridging solvent. (**C**) The structure of the guanylylpyridone (in its 2-pyridinol tautomeric form) as determined by NMR and mass spectrometry in the enzyme-free state (*23*). (**D**) Fourier-transformed EXAFS of [Fe]-hydrogenase as crystallized. The peaks at 1.8 Å (C) and 2.9 Å (O) are dominated by the CO contribution. The fit is, at best, compatible with the presence of two COs, one N/O, one S ligand, and of another not fully occupied first-shell ligand (see table S1).

One iron ligand is the pyridinol nitrogen atom, which links the organic guanylylpyridinol molecule with the iron center (Fig. 4). The hydroxylate and carboxylate substituents of pyridinol are not used as iron ligands, although the latter is partly disordered and must be analyzed with caution. Because of its planarity, the heterocyclic ring is present in a pyridinol and not in a pyridone tautomeric form (Fig. 4C), and therefore the nitrogen atom is in a π-accepting sp^2 configuration. The pyridinol group—in particular, if the hydroxyl group is in a deprotonated state—might have ligand back-bonding properties similar to those of cyanide (*29*, *30*), which acts as an iron ligand in the [NiFe]- and [FeFe]-hydrogenases. Further ligands are two CO molecules that are optimally accommodated between several nonpolar atoms of the polypeptide chain. The CO molecules form an angle of 90°, in agreement with the interpretation of the IR spectrum of the holoenzyme (*24*). The sole proteinaceous ligand originates from the thiolate sulfur of Cys^{176} that points toward the iron from a loop following strand 169:174 at the bottom of the intersubunit cleft.

The chemical nature of the fifth ligand is unknown, and its electron density cannot definitely be assigned as a monatomic or diatomic ligand, although it is clearly connected with that of the iron and of relatively high occupancy (i.e., corresponds to a completely occupied water molecule). The electron density (σ = 1.2) at the position of the unknown ligand (fig. S2) is increased (by a factor of 1.6) after soaking of the crystals with 3 mM cyanide, which suggests that this is the binding site of the reversible and noncompetitive inhibitor cyanide (*22*).

The vacant sixth coordination site of the iron contains a spherical electron density interpreted as a monatomic solvent molecule (i.e., a completely occupied water molecule) that is, however, at a distance of 2.7 Å too far away to be considered as a ligand (Fig. 4). We predict this site to be the binding position of extrinsic CO (Fig. 5) known to inhibit [Fe]-hydrogenase, as the Fourier-transformed IR spectrum of the CO inhibited enzyme predicts a perpendicular orientation of the extrinsic CO relative to the two intrinsic COs (*24*). Because CO is a competitive inhibitor with respect to H_2, the latter most likely also binds to this site (Figs. 1 and 5). The solvent close to the iron interacts with a second solvent molecule, which in turn is linked to the carbonyl group of the strictly conserved Cys^{250} (Fig. 4A). Interestingly, the Cys^{250} → Ala mutant shows reduced enzyme activity (table S2).

A comparison of the active-site metal centers of the three hydrogenase types reveals unexpected common features (Fig. 1) previously recognized for the two binuclear hydrogenases. All three types contain a redox-inactive low-spin iron, presumably in the oxidation state II, that is asymmetrically ligated by five or six ligands arranged as a distorted square pyramid or octahedron. Moreover, three π-accepting ligands comprising CO, cyanide, or pyridinol (considered as a cyanide functional analog) are oriented perpendicular to each other in a geometrically related manner, and a thiolate sulfur always coordinates the iron trans to a diatomic molecule (Fig. 1). All three iron centers act together with a redox-active partner—methenyl-H_4MPT^+ in the case of [Fe]-hydrogenase, the distal iron in the case of [FeFe]-hydrogenase, and nickel in the case of [NiFe] hydrogenase—whose spatial position relative to the other ligands is also similar. Apparently, these related iron centers, with unusual nonproteinaceous ligands thought to be synthesized by three different enzymatic machineries (*31*) and embedded into three architecturally different hydro-

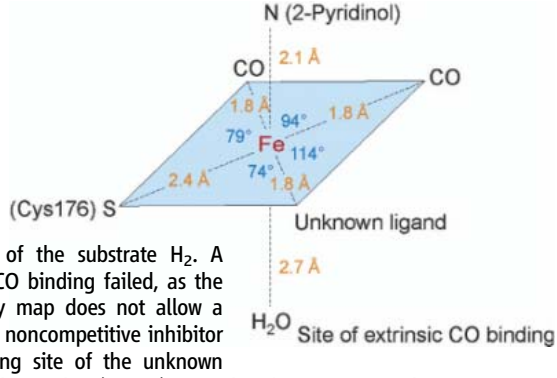

Fig. 5. Coordination of the iron in the active site of [Fe]-hydrogenase. The iron sits in the plane of the square spanned by the Cys^{176} sulfur, two COs, and the unknown ligand; the nitrogen is at the top of the pyramid. The sixth ligation site trans to the pyridinol nitrogen is presumably the binding site of the competitive inhibitor CO (according to IR spectroscopy data) and of the substrate H_2. A structural confirmation of the extrinsic CO binding failed, as the quality of the resulting electron density map does not allow a distinction between a water and CO. The noncompetitive inhibitor cyanide presumably occupies the binding site of the unknown ligand as derived from x-ray crystallographic studies (fig. S2). Note that the iron, one of the intrinsic COs, the unknown ligand (or the cyanide), and probably the extrinsic CO are located in the plane of the pyridinol ring. The distances and angles between the irons and ligands are drawn in, except for those between the four ligands in the plane and the pyridinol's nitrogen and the nearby solvent assigned as water [N/S, 86°; N/CO (left), 102°; N/CO (right), 91°; N/cyanide, 94°; H_2O/S, 90°; H_2O/CO (left), 72°; H_2O/CO (right), 91°; H_2O/cyanide, 90°]. The distances between iron and the four identifiable ligands agree well with those determined in parallel by EXAFS spectroscopy (Fig. 4C and table S1).

genase structures, evolved independently. Remarkably, hydrogenases are the only metalloenzymes that use toxic CO and cyanide (or pyridinol) as metal ligands. Thus, hydrogenases are an impressive example of convergent evolutionary development as a consequence of specific biological and/or chemical restraints. However, the intrinsic physicochemical properties of the unique iron ligation pattern are not yet understood, nor are their implications for the technologically important H_2 activation reaction.

Despite the related low-spin iron centers, the enzymatic mechanism of [Fe]-hydrogenase differs fundamentally from that of the other types because of the different nature of the redox-active partner and the accompanying electron delivery mode. In [NiFe]- and [FeFe]-hydrogenases, the electrons of H_2 reduction flow one by one through the redox-active metals and several iron-sulfur clusters over a large distance to an electron acceptor. The active-site structures essentially remain fixed during H_2 cleavage, and H_2 reaches the deeply buried active site by a long diffusion channel. In [Fe]-hydrogenase, however, the found ternary reaction mechanism and the exchange between H_2 and protons of water solely in the presence of methenyl-H_4MPT^+ (*32*) (see partial structure in Fig. 2) suggests that methenyl-H_4MPT^+ directly accepts the hydride from H_2. This conclusion is supported by the x-ray structure, as the cleft between the peripheral and central units can accommodate the bulky methenyl-H_4MPT^+ molecule and the C14a atom can be positioned sufficiently close to the iron without causing severe clashes with the polypeptide chain (fig. S3). Because the intersubunit cleft in the holoenzyme is, in fact, too large for an optimal methenyl-H_4MPT^+ adjustment, we assume that its binding is accompanied by an induced-fit movement constituting the catalysis-competent active-site before each turnover. The expected large-scale conformational changes are reflected in the different positions of the peripheral unit relative to the central unit found in the structures of the holo- and apoenzymes (Fig. 3), mainly induced by crystal forces. H_2 can readily reach the solvent-exposed Fe center, which is probably encapsulated upon methenyl-H_4MPT^+ binding.

The most attractive hypothesis for the mechanism of H_2 cleavage in [Fe]-hydrogenases is based on a concerted action of the strong hydride acceptor methenyl-H_4MPT^+ and the Lewis acid Fe(II) that lowers the pK_a value of H_2, preferably when bound in a side-on conformation. The polarized H_2 ligated to the postulated binding site (Fig. 5) is attacked from the adjacent carbocation C14a of methenyl-H_4MPT^+ from the *Re*-face of the ring system (see Fig. 2), generating methylene-H_4MPT. Acceptors for the released proton within 6.5 Å from the iron include the Cys^{176} thiolate ligand, the pyridinol nitrogen, oxygen, and carboxyl oxygen as well as two conserved histidines, His^{14} and His^{201} (for the position of the two histidines relative to the iron, see fig. S1B). A $His^{14} \rightarrow$ Ala mutation drastically reduces the hydrogenase activity of the enzyme, whereas $His^{201} \rightarrow$ Ala has only a minor effect (table S2).

Although there are still many questions to be answered, the crystal structure allows us to draw the following conclusions: (i) The active-site iron is definitely mononuclear, not dinuclear as in the [FeFe]- and [NiFe]-hydrogenases. (ii) The presented structural data, together with results of studies using various spectroscopic methods [nuclear magnetic resonance (NMR), mass, IR, Mössbauer, and extended x-ray absorption fine structure (EXAFS) (*23*–*26*)] and information from mutational analysis (*25*), converge to a coherent result. (iii) The structures of the [Fe]-, [FeFe]-, and [NiFe]-hydrogenases are completely different but share features in their active site that can only have evolved convergently (Fig. 1). (iv) The detailed three-dimensional structure will allow density functional theory (DFT) calculations of energy profiles, which will help to exclude some of the proposed mechanisms of H_2 activation. (v) Model complexes can be constructed on the basis of the iron center of [Fe]-hydrogenase, and their analysis will provide further insight into its essential but not yet understood function in H_2 activation (*3*–*7*).

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33. Supported by the Max Planck Society, the Fonds der Chemischen Industrie, and the Bundesministerium für Bildung und Forschung ($BioH_2$ project). We thank H. Michel for continuous support, and the staff of beamline PXII at the Swiss Light Source for help during data collection. Coordinates of [Fe]-hydrogenase and [Fe]-hydrogenase complexed with cyanide have been deposited in the Research Collaboratory for Structural Bioinformatics Protein Data Bank under accession codes 3DAG and 3DAF.

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10 April 2008; accepted 5 June 2008
10.1126/science.1158978

Manipulating the Metazoan Mitochondrial Genome with Targeted Restriction Enzymes

Hong Xu, Steven Z. DeLuca, Patrick H. O'Farrell*

High copy number and random segregation confound genetic analysis of the mitochondrial genome. We developed an efficient selection for heritable mitochondrial genome (mtDNA) mutations in *Drosophila*, thereby enhancing a metazoan model for study of mitochondrial genetics and mutations causing human mitochondrial disease. Targeting a restriction enzyme to mitochondria in the germline compromised fertility, but escaper progeny carried homoplasmic mtDNA mutations lacking the cleavage site. Among mutations eliminating a site in the cytochrome c oxidase gene, *mt:CoI*^{A302T} was healthy, *mt:CoI*^{R301L} was male sterile but otherwise healthy, and *mt:CoI*^{R301S} exhibited a wide range of defects, including growth retardation, neurodegeneration, muscular atrophy, male sterility, and reduced life span. Thus, germline expression of mitochondrial restriction enzymes creates a powerful selection and has allowed direct isolation of mitochondrial mutants in a metazoan.

A typical animal cell contains hundreds to thousands of copies of the mitochondrial genome (mtDNA), which encodes 13 essential subunits of the electron transport chain complexes and RNAs (2 rRNAs and 22 tRNAs) required for mitochondrial translation (*1*, *2*). It is not clear how the genetic integrity of this amitotically distributed genome is main-

tained. Due to a high mutation rate, lack of recombination, and the presence of coresident genomes to protect mutant genomes from selection, mtDNA mutations should readily accumulate in cells (*3*). Indeed, a dramatic accumulation of mitochondrial mutations in somatic tissues appears to contribute to age-related disorders in humans (*4*), and numerous mutations on human mtDNA have been linked to maternally inherited diseases (*1*, *2*). The mitochondrial genome has not been very amenable to functional genetic studies in metazoans (*5*, *6*). Successful generation of trans-mitochondrial mice by hybridizing mtDNA-depleted embryonic stem cells and enucleated donor cells containing mtDNA mutations (*6*) has been constrained by the limited collections of donor cells carrying mtDNA mutations.

Targeting restriction enzymes to mitochondria can selectively compromise propagation of mtDNA that has a site targeted by the enzyme (*7*). We reasoned that expression of a restriction enzyme targeting wild-type mtDNA would create a selection allowing rare mutations lacking the restriction site to take over (fig. S1). XhoI has a single recognition site in the cytochrome c oxidase subunit I locus (*mt:CoI*) in the *Drosophila melanogaster* mitochondrial genome (fig. S2). It overlaps codons specifying three amino acid residues in a conserved region of CoI (Fig. 1E and fig. S3). *pMT-mitoXhoI*, which encodes XhoI fused to a mitochondrial targeting leader at its N terminus and a Myc tag at its C terminus (fig. S4), was used to establish S2 cell lines. MitoXhoI protein was specifically localized to mitochondria (fig. S4). Upon induction, the majority of the mtDNA was cleaved at the XhoI site (Fig. 1, A and B), demonstrating that MitoXhoI can efficiently target the wild-type mitochondrial genome.

To test the consequence of MitoXhoI expression in animals, we generated transgenic flies expressing *mitoXhoI* under the UASp promoter. Ubiquitous expression of *UAS-mitoXhoI* activated by a *tub-GAL4* driver blocked hatching of 95% of the embryos, and the remaining 5% died as first instar larvae. Selective expression of *UAS-mitoXhoI* in the eye primordium using an *eyeless-GAL4* driver ablated or greatly reduced the eye (Fig. 1C).

To select for heritable mitochondrial mutants, we used *nanos-GAL4* to activate *UAS-mitoXhoI* expression in germline cells, reasoning that those cells carrying XhoI-resistant mtDNA would survive to produce progeny carrying the mutant mtDNA (fig. S5). Most of the *UAS-mitoXhoI*/+; *nanos-Gal4*/+ flies were sterile, but about 1% of the females gave a few escaper progeny. Escapers all carried XhoI-resistant mtDNA (Fig. 1D), and no wild-type genomes were detected, which suggests that each mutant was homoplasmic. Sequencing identified single base pair mutations that eliminated the XhoI site (Fig. 1E). Three different sequence variants were recovered, *mt:CoI*^{A302T}, *mt:CoI*^{R301L}, and *mt:CoI*^{R301S}, all resulting in a single amino acid change (Fig. 1E). No secondary mutations were seen in these mutants, and each mutant was maternally inherited and conferred complete resistance to MitoXhoI in the eye (Fig. 1C).

We investigated whether any of these *mt:CoI* mutations were pathogenic. Only *mt:CoI*^{A302T} behaved as wild-type flies in the phenotypic analyses we conducted (table S1). *mt:CoI*^{R301L} flies were healthy, except that the males were sterile (table S1). Spermatogenesis appeared normal, and sperm were motile but reduced in number (movies S1 and S2). Sperm were transferred to the female during mating but were not stored in the sperm storage organs. Despite being sterile, male *mt:CoI*^{R301L} flies had similar cytochrome c oxidase activity and slightly higher adenosine triphosphate (ATP) levels than wild-type flies (fig. S6). It is not clear how a mitochondrial mutation selectively compromises sperm function, but, because transmission of mtDNA occurs through the female, it has been argued that such mutations can be sustained in a population (*8*) and could make a substantial contribution to male sterility (*9*). The *mt:CoI*^{R301S} allele exhibited markedly more severe phenotypes. The mutant had an extended larval phase (about 10 days at 25°C), and the mechanosensory bristles on the thorax were missing or thinned and shortened (Fig. 2, A and B). The males were sterile without any mature sperm, which suggests a defect in spermatogenesis. Female flies were fertile but produced only 20% as many progeny as did wild-type flies. The *mt-CoI*^{R301S} flies had about half the normal cytochrome c oxidase activity and significantly reduced ATP levels (fig. S6).

Because most mtDNA diseases in humans show neuropathy and myopathy, we examined the morphology of retina and indirect flight muscles to determine whether *mt:CoI*^{R301S} flies had similar defects. Young *mt:CoI*^{R301S} flies had the full complement of ommatidia components, although some rhabdomeres had slight morphogenetic defects (Fig. 2, C and D). However, the ommatidia of aged *mt:CoI*^{R301S}, but not wild-type flies, were disorganized and the rhabdomeres were shrunken or completely lost (Fig. 2E), indicating age-dependent degeneration of photoreceptor neurons.

Transmission electron microscopy (TEM) of flight muscle in wild-type and young *mt:CoI*^{R301S} flies revealed orderly muscle fibers and fused mitochondria with long and tubular cristae (Fig. 2, F and G). However, two weeks after adult eclosion, the *mt:CoI*^{R301S} mitochondria were small and fragmented, contained many vesicular structures, and

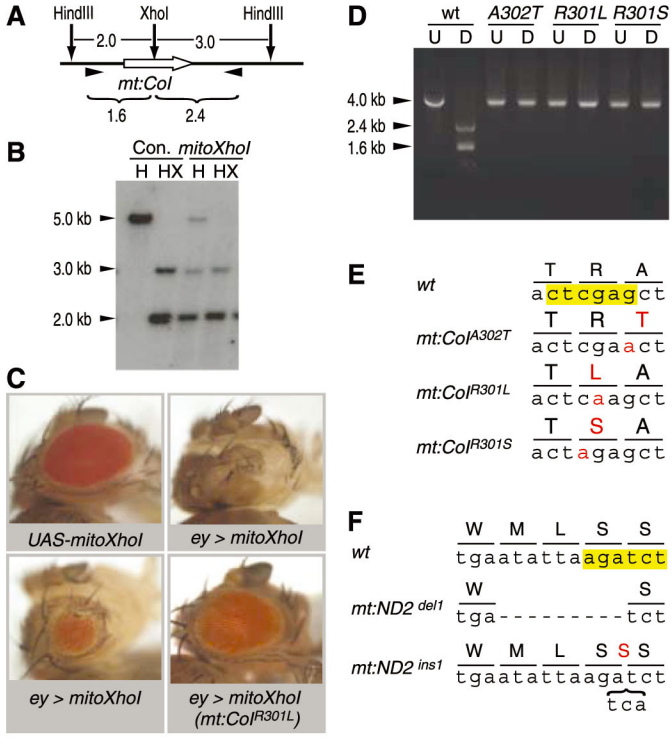

Fig. 1. Manipulation of mtDNA with a mitochondrially targeted XhoI (MitoXhoI). (**A**) Restriction map of a mitochondrial genomic region spanning cytochrome c oxidase subunit 1 (*mt:CoI*, thick white arrow). The size of each fragment is given in kb. (**B**) Total DNA was extracted from control (Con.) and *mitoXhoI*-expressing (*mitoXhoI*) S2 cells, digested with HindIII (H) or HindIII and XhoI (HX), and hybridized with probe against the *mt:CoI* gene. (**C**) *UAS-mitoXhoI*, when expressed under the control of *eyeless-Gal4* (*ey>mitoXhoI*), caused eye ablation or small eye, which was suppressed in flies containing XhoI-resistant mtDNA [*ey>mitoXhoI(mt:CoI*^{R301L})]. (**D**) mtDNAs were amplified using the pair of primers denoted as arrowheads in (A). U, undigested polymerase chain reaction (PCR) product; D, PCR product digested with XhoI. There are no detectable wild-type bands in any of the mutants. (**E**) Recovered mutant mtDNAs with mutations in the XhoI site (yellow). Mutated nucleotides and changed amino acid residues are highlighted in red. (**F**) Recovered mutant mitochondrial genomes with mutations affecting the BglII site (yellow). Dashed line represents a 9-nucleotide deletion. Bracket represents a 3-nucleotide insertion encoding an extra serine residue (red).

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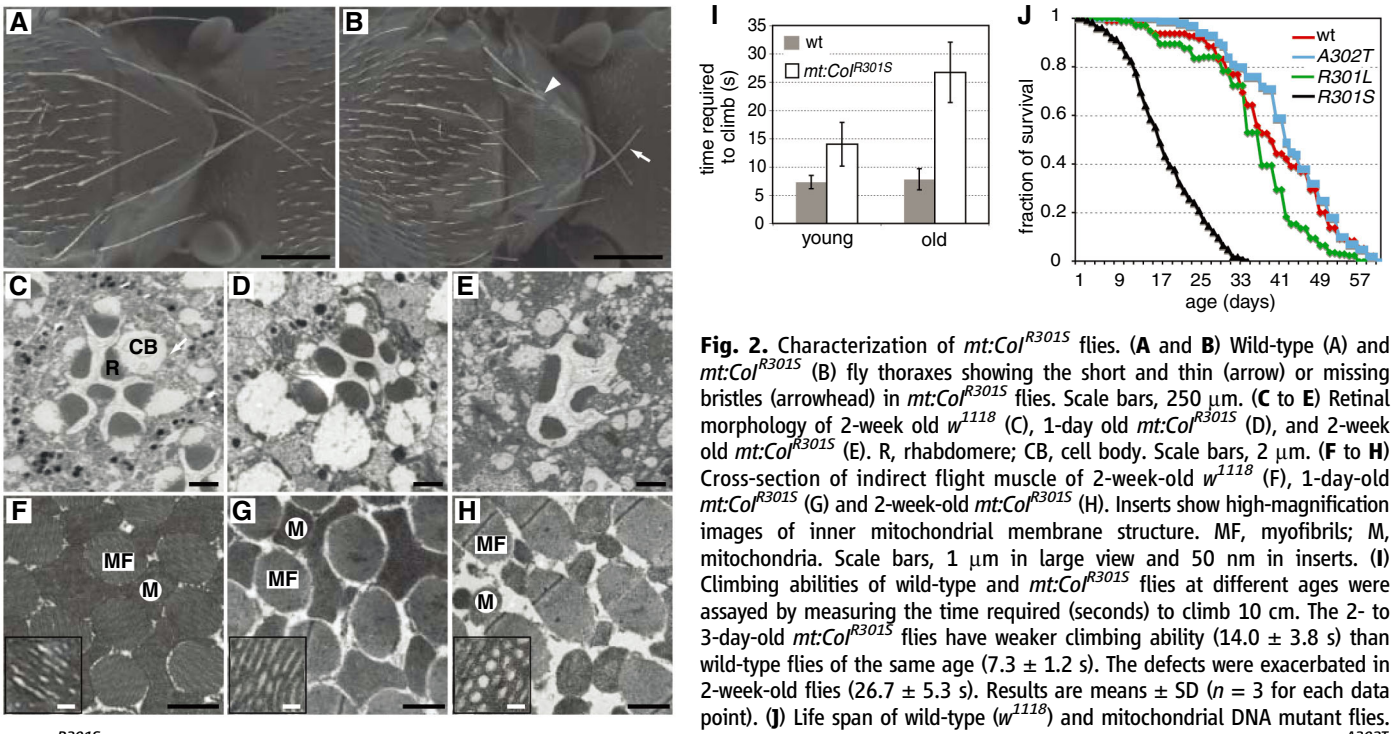

Fig. 2. Characterization of *mt:CoI*^{R301S} flies. (**A** and **B**) Wild-type (A) and *mt:CoI*^{R301S} (B) fly thoraxes showing the short and thin (arrow) or missing bristles (arrowhead) in *mt:CoI*^{R301S} flies. Scale bars, 250 μm. (**C** to **E**) Retinal morphology of 2-week old w^{1118} (C), 1-day old *mt:CoI*^{R301S} (D), and 2-week old *mt:CoI*^{R301S} (E). R, rhabdomere; CB, cell body. Scale bars, 2 μm. (**F** to **H**) Cross-section of indirect flight muscle of 2-week-old w^{1118} (F), 1-day-old *mt:CoI*^{R301S} (G) and 2-week-old *mt:CoI*^{R301S} (H). Inserts show high-magnification images of inner mitochondrial membrane structure. MF, myofibrils; M, mitochondria. Scale bars, 1 μm in large view and 50 nm in inserts. (**I**) Climbing abilities of wild-type and *mt:CoI*^{R301S} flies at different ages were assayed by measuring the time required (seconds) to climb 10 cm. The 2- to 3-day-old *mt:CoI*^{R301S} flies have weaker climbing ability (14.0 ± 3.8 s) than wild-type flies of the same age (7.3 ± 1.2 s). The defects were exacerbated in 2-week-old flies (26.7 ± 5.3 s). Results are means ± SD (n = 3 for each data point). (**J**) Life span of wild-type (w^{1118}) and mitochondrial DNA mutant flies. *mt:CoI*^{R301S} flies showed greatly reduced longevity, with a median life span of 17 days. The median life span of wild-type flies is 41 days. Neither *mt:CoI*^{A302T} nor *mt:CoI*^{R301L} flies displayed any significant alterations in life span compared with wild-type flies.

did not completely fill the intermyofibril space (Fig. 2H). Similar mitochondrial morphological defects were also reported in flies with a mutation in the mitochondrial *ATP6* locus (*10*). Moreover, when tested in a climbing assay, *mt:CoI*^{R301S} flies showed mobility defects enhanced by age, consistent with age-dependent neurodegeneration and myopathy (Fig. 2I). Beyond these age-dependent neurological and muscular dysfunctions, *mt:CoI*^{R301S} flies had substantially reduced longevity (Fig. 2J).

The pathologies associated with human mitochondrial DNA diseases often develop with age (*1*), much like the degenerative changes that we find for the *mt:CoI*^{R301S} allele. Because the mutations responsible for human mitochondrial disease usually affect only a fraction of the mitochondrial genomes (heteroplasmy), the progressive increase in the severity of the phenotype might be attributed to an increase in the load of mutant genomes in the affected tissues or to an inherent feature of the mitochondrial defect. Because the mutations we have selected are homoplasmic, the age-dependent degenerative phenotype must be inherent to the mutation.

There are 31 single-cutting restriction enzymes sites in *Drosophila* mtDNA, distributed within eight protein-encoding genes, two rRNAs and three tRNAs (table S2). To test the generality of our approach, we produced a *UASp*-regulated *mitoBglII* transgene that targets a single BglII site in the *mt:ND2* locus. After germline expression, we recovered two lines carrying mutations in *mt:ND2*. In contrast to the single base changes recovered in *mt:CoI*, one *mt:ND2* mutation is an in-frame deletion and the other is a three-nucleotide insertion (Fig. 1F). Use of the full panel of available enzymes will target the majority of the protein coding genes in *Drosophila* mtDNA and allow generation of multiple alleles, although we expect that alleles that completely eliminate respiratory activity will not be recovered as a result of lethality.

In the described selection for resistance to germline expression of mitochondrial restriction enzymes, roughly one out of several thousand germline precursor cells (PGCs) survived to produce progeny. Mice have about 50 PGCs, and this population is greatly expanded before definitive germ cell differentiation (*11*). Furthermore, one could employ the previously developed "mtDNA mutator mouse" carrying an error-prone mitochondrial DNA polymerase (*12*) to increase the frequency of enzyme-resistant mitochondrial genomes. If the frequency of mtDNA mutations in mice is similar to that in flies, generation of novel homoplasmic mitochondrial mutant mice by the described approach should be practical.

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13. We thank members of O'Farrell laboratory for comments on the manuscripts; M.-L. Wong and M. Sepanski for technical help with TEM; M. Fuller and A. W. Shermoen for help with examination of spermatogenesis and sperm in female reproductive organs; and Bloomington Stock Center for providing some *Drosophila* stocks. This work is supported by an NIH RO1 grant and a Sandler Family Supporting Foundation award to P.H.O. H.X. is supported by a postdoctoral fellowship from the Larry L. Hillblom Foundation.

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The Medical College of Georgia (MCG), in partnership with the University of Georgia (UGA) is seeking a senior basic scientist and educator for the newly established position of Chair, Department of Basic Sciences. The Basic Sciences Chair will be charged with building an integrated and collaborative medical school basic sciences phase 1 & 2 curriculum, and with fostering collaborative research that bridges and builds upon the strengths of the partner institutions. The successful candidate will participate in shaping the future of the new 4-year medical school campus of the MCG and UGA in Athens, GA. The Athens campus faculty will be linked to the MCG main campus in Augusta by administrative support and teaching technology. Projections for LCME accreditation could seat the first class no later than 2010.

MCG and UGA are two of four research-intensive universities of The University System of Georgia, funded by state higher education dollars. The MCG School of Medicine and the University of Georgia both enjoy significant extramural support for their missions of education, research and service, and both have engaged with health systems and physicians, and local community groups, to create a model of 21st century medical education excellence.

The successful candidate for Chair, Department of Basic Sciences of the MCG/UGA medical education partnership will be an outstanding scientist of national reputation, preferably in one or more of the research strategic foci of physiology; proteomics and genomics; pharmacology and pharmaco-genomics; cell biology; epidemiology; immunology/immunotherapy; and pathogenesis of infectious diseases. Although it is also anticipated that the Basic Sciences Chair will have a research interest in cancer, diabetes, or cardiovascular disease, as the leading causes of death in Georgia, other relevant areas of research expertise will be welcomed.

Minimum requirements include a MD, PhD, or MD/PhD with minimum of five years of experience as an associate professor or equivalent. A record of sustained academic accomplishments and evidence of leadership in his/her field, relevant administrative experience, and evidence of effective interpersonal, collaborative and communication skills are required. The successful candidate is expected to have a distinguished record of scholarly activity, a history of extramural funding, and experience in a medical/graduate education curriculum. A track record of building interdisciplinary programs through faculty development and recruitment, and with the successful mentoring of medical students is also highly desirable.

Please submit your curriculum vitae to:

R. Clinton Webb, Ph.D.
H.S. Kupperman Chair in Cardiovascular Disease
Professor and Chairperson
Department of Physiology, Medical College of Georgia
1120 Fifteenth Street, Augusta, GA 30912
or to: **cwebb@mcg.edu**

Universität Hamburg

At the **Research Unit Sustainability and Global Change** – a joint project of the **Faculty for Mathematics, Informatics and Natural Science and the Faculty for Economics and Social Sciences** – the position of a

UNIVERSITY PROFESSOR (W 3)

for **„Sustainability and Global Change"**

is offered starting as soon as possible. **(Code 1989/W3)**

The University of Hamburg aims at increasing the number of women as scientific staff and therefore specifically requests applications from qualified women for this position.

Areas of Responsibility:
Representation of this field in both research and teaching. Successful candidates are expected to carry out excellent research in the field of scientific and socio-economic modelling of environmental change and its consequences. The development of large and small scaled models is desired. A central responsibility will be the further development of Hamburg's interdisciplinary research on the topic of sustainability and the intensification of cooperation between scientists from the natural, economic and social sciences, particularly within the scope of the Cluster of Excellence „Integrated Climate System Analysis and Prediction (CliSAP)". An important aspect will be the initiation and fertilisation of a discourse with stakeholders outside of the university with regard to sustainable development at the regional as well as the global levels. The Professor will receive substantial support from the Cluster of Excellence, CliSAP, and will be expected to set milestones for sustainability research focusing on climate impacts.

Personal Qualifications:
We seek highly motivated candidates with an outstanding track record in sustainability research based on natural science and socio-economics. Their education should be either in natural, economic or social science with experience in the other areas of expertise. In addition, an exceptionally well-developed talent for networking and cooperation as well as very good communication skills are desired. In addition to their scientific credentials, the candidates are expected to have especially good didactic capabilities and experience in teaching. Our goal is to incorporate education on the topic of sustainability into the study courses of the natural, economic and social sciences at the University of Hamburg.

Teaching obligations:
According to the Hamburg „Lehrverpflichtungsverordnung". As compensation for particularly active and successful research, a reduction in teaching obligations may be taken into consideration.

Basic requirements:
According to § 15 Hamburgisches Hochschulgesetz.

Disabled persons are given priority over applicants of equal suitability, qualification and degree of specialized knowledge.

Foreign candidates will be expected to be proficient in the German language or to be willing to learn it quickly.

Applications (including a current CV, a list of publications, a list of previously taught courses and a concept for future research and teaching) should be sent under Code 1989 W3 to: The President of the University of Hamburg, Referat Organisation & Personalentwicklung -631.6-, Moorweidenstraße 18, 20148 Hamburg, Germany.

The deadline for receipt of applications is **September 5th, 2008**.

The University of Hamburg is an equal opportunity employer.

TOR ZUR WELT DER WISSENSCHAFT

NIMH National Institute of Mental Health

NEUROSCIENCE/GENETICS POSITION AVAILABLE

Are you interested in an exciting, meaningful and challenging career working with some of the most outstanding scientists in the world? Then the National Institute of Mental Health (NIMH), a major research component of the National Institutes of Health (NIH) and the Department of Health and Human Services, invites you to send a letter of interest for the position of Health Scientist Administrator in the Division of Extramural Activities, Extramural Review Branch (ERB).

NIMH supports innovative science that will profoundly transform the diagnosis, treatment, and prevention of mental disorders, paving the way for a cure. The Extramural Review Branch within DEA is responsible for moving the science forward through management of the initial review of grant applications in a range of scientific disciplines. Along with this responsibility, the successful candidate for this position will have the opportunity to work with other scientists at NIMH and across the country in moving research forward through development of new initiatives, workshops, and conferences. Come join a team of people passionate about improving the lives of individuals suffering with mental illness!

Mandatory Selection Criteria: For expertise in neuroscience, the criteria are knowledge of research pertaining to the causes, diagnosis, and treatment of mental illness affecting all ages and socio-cultural groups (e.g., etiology, epidemiology, assessment, development/efficacy/effectiveness of psychosocial and/or pharmacologic interventions). In addition, the applicant is expected to have knowledge of principles, theories, and research methods in areas such as pharmacology, psychiatry, brain imaging, neurodevelopment, and/or molecular/ statistical genetics. Experience with clinical populations would be helpful. In order to qualify for this career position you should have a Ph.D. and/or M.D. degree in a relevant field of biomedical or behavioral science and appropriate experience in one of the above stated areas. Appointees must be a U.S. citizen. Salary is commensurate with experience and expertise.

Your letter of interest for this position, including a brief description of career interests, a curriculum vitae and a bibliography, should be submitted to: **Dr. Henry Haigler c/o Ms. Stephanne Player, 6001 Executive Blvd., Room 6166, Bethesda, MD 20892-9609; E-mail: players@mail.nih.gov; Fax: (301) 480-3402**. For information concerning the nature of the position, contact **Dr. Henry Haigler at (301) 443-7216 or E-mail hhaigler@mail.nih.gov**. **All letters of interest are due no later than August 29, 2008**. The Institute has a strong commitment to the diversity of its workforce and a biomedical research environment that reflects the diversity of the American population (**http://oeo.od.nih.gov/**).

NIEHS National Institute of Environmental Health Sciences

Department of Health and Human Services
National Institutes of Health (NIH)
Director, National Institute of Environmental Health Sciences (NIEHS)

THE POSITION: The NIH is seeking exceptional candidates for the position of Director, NIEHS, to provide leadership to one of the preeminent centers for environmentally-related research in the world. The Director, NIEHS, also serves in a dual role as the Director, National Toxicology Program, and in this role reports to the Secretary, Department of Health and Human Services (DHHS). This position offers a unique opportunity for the right individual to provide strong and visionary leadership to an organization dedicated to reducing the burden of human illness and dysfunction from environmental causes by understanding each of these elements and how they interrelate. The Director will manage a high-level complex organization and must demonstrate integrity and fairness, adhering in work and behavior to the highest ethical standards, and upholding the highest standards of scientific research and/or business practices. Applicants must possess an M.D. and/or Ph.D. and have senior-level research experience and knowledge of research programs in one or more scientific areas related to environmental effects on human health and/or toxicology research. They should be known and respected within their profession, both nationally and internationally, as individuals of outstanding scientific competence. Salary is commensurate with experience, and full Federal benefits, including leave, health and life insurance, retirement and savings plan (401K equivalent) will be provided. A detailed vacancy announcement that includes application procedures is available at **http://www.jobs.nih.gov (under Executive Jobs)**. **NIEHS is located in Research Triangle Park (RTP), North Carolina**. Questions may be addressed to **Ms. Lynnita Jacobs at: SeniorRe@od.nih.gov**. CV and bibliography must be received by **11:59 p.m. Monday, August 4, 2008.**

UNIVERSITY OF Cincinnati

Cincinnati Children's Hospital Medical Center

University of Cincinnati College of Medicine
Department of Cancer and Cell Biology - Tenured/Tenure Track Faculty Positions in Cancer and Inflammation Research

As part of building the newly created Department of Cancer and Cell Biology at the University of Cincinnati College of Medicine, Cincinnati Children's Hospital Medical Center, the University Hospital, and the College of Medicine are pleased to announce a Joint Initiative in Cancer Biology Research. We seek applications for tenured/tenure-track faculty positions at all ranks from exceptional researchers with documented accomplishments in the area of inflammation as it relates to the development of various cancers. Strong preference will be given to applicants with a focus on inflammatory processes that contribute to the development of lung, breast, and prostrate cancers. Applicants should hold a Ph.D., M.D. or equivalent degrees and have an outstanding record of publications with demonstrated commitments to pursuing cutting-edge research, and graduate education. Candidates at the Associate or Full Professor level are expected to have an outstanding, externally funded, research program.

The successful candidates will join the Department of Cancer and Cell Biology whose current faculty members lead highly visible research programs related to defining the signal transduction pathways central to the development of various types of cancer including prostate, lung, breast, endometrial, and kidney cancer. Candidates will have the opportunity to forge strong interdisciplinary and translational research collaborations with outstanding campus wide programs in cancer biology, gene therapy, molecular genetics, hematology, and immunology. The Department has vibrant graduate and postgraduate training programs with ~45 graduate students supported by NIH training grants and State funds. Outstanding facilities are available including state-of-the-art research facilities and multiple research cores (transgenic/gene knockout; microarray/ bioinformatics; proteomics; genotyping; flow cytometry and high-speed cell sorting; high-throughput drug screening; viral vector production; and biostatistical analysis). Detailed information can be accessed through **http://cnal.uc.edu/monc**.

These positions offer competitive salaries and generous start-up packages commensurate with the candidate's qualifications. Cincinnati, including its suburbs, is a beautiful and affordable city with many cultural attractions. Qualified individuals can apply by submitting a CV, statement of research interests and names of referees to **www.jobsatuc.com** (position **#28UC0816**) or via email to **Dr. Jorge Moscat, Chairman, Cancer and Cell Biology Department** at **cancercellrecruit@uc.edu**.

Texas A&M Health Science Center College of Medicine

Faculty Positions in the Division of Molecular Cardiology Department of Medicine

Several tenure-track, State of Texas funded positions at the Assistant and Associate Professor levels are available. Focus of the Division is Cardiovascular, with emphasis on cardiac hypertrophy, remodeling and heart failure. Funded candidates with a molecular approach that interfaces with translational initiatives are encouraged to apply. Competitive startup packages, incentives and lab space are available. Core facilities include microarray, imaging (confocal and atomic force), proteomics, cell sorting and analysis, and laser capture microdissection. The Division, which is part of the Texas A&M College of Medicine, is located in Temple, Texas, a vibrant city close to Austin.

In addition to research, new faculty will instruct fellows and residents, medical and/or graduate students, and will be active in pre- and postdoctoral training. Interactions with clinical researchers are encouraged through Scott & White and the Central Texas Veterans Health Care System, major teaching hospitals of the Texas A&M Health Science Center. The College of Medicine is entering a rapid growth phase with expansion of the medical school class, and a substantial number of new faculty will be recruited in upcoming years.

Send CV, statement of research, training and teaching goals, and a list of 3 references to: **kbaker@medicine.tamhsc.edu** or **Kenneth M. Baker, M.D., Division of Molecular Cardiology, Texas A&M Health Science Center, College of Medicine, 1901 South First Street, Building 205, Temple, Texas 76504**.

Drexel UNIVERSITY COLLEGE OF MEDICINE

DEPARTMENT OF PHARMACOLOGY & PHYSIOLOGY

Assistant/Associate Professor

The Department of Pharmacology and Physiology, Drexel University College of Medicine, invites applications from qualified individuals with a Ph.D., M.D. or equivalent degree for a tenure-track educator faculty position at the Assistant or Associate Professor level. We seek an individual to serve as a medical educator who will be enthusiastically committed to the teaching of physiology to medical and graduate students. Applicants with interests in curriculum development and innovative teaching methods are especially encouraged to apply. Drexel University College of Medicine has two medical school curricula consisting of an integrated and a problem-based program. The individual filling this position will be the course director for the problem-based learning program. There are opportunities to use simulation techniques and to facilitate small groups within the medical curriculum. Drexel University College of Medicine has separate tenure and promotion criteria for medical educators, clinicians, and researchers. Criteria for promotion and tenure in the educator track are based on excellence in teaching as well as scholarly activity including publications and research that preferably deals with educational approaches to the teaching of physiology. The department has graduate programs in Pharmacology and Physiology and in Drug Discovery leading to the MS and Ph.D. degrees.

For more information please consult the following websites for Department of Pharmacology and Physiology (**http://www.drexelmed.edu**;) and on medical education at Drexel University College of Medicine (**http://webcampus.drexelmed.edu/**).

Applicants should submit curriculum vitae, a statement of teaching philosophy and teaching interests, and the names of three references to **Carolann.Imbesi@Drexelmed.edu**. Review of applications will begin immediately and continue until the position is filled.

MAX-PLANCK-INSTITUT FÜR BIOPHYSIKALISCHE CHEMIE
KARL-FRIEDRICH-BONHOEFFER-INSTITUT GÖTTINGEN

Postdoctoral Positions
at the Max Planck Institute for Biophysical Chemistry, Göttingen (Code Number 16-08)

Potential projects will integrate genetic, cell biological, behavioral, histological and molecular experimental approaches:

I. Regulation of stem cell division and maintenance by the RNAi pathway in *Drosophila* germline (Shcherbata HR et al 2007 ***Cell Stem Cell;*** Hatfield SD, Shcherbata HR et al 2005 ***Nature).***
We will try to figure out stage-specific requirements for Dicer-1 and Dicer-2 dependent pathways for germline stem cell maintenance and division.

II. A *Drosophila* model of muscular dystrophy (Shcherbata HR et al 2007 ***EMBO J).***
After successfully performed genetic screens we identified modifiers of Dg-Dys complex defects. Further analysis of these interactors will provide new insights into the origin of muscular dystrophy and potential treatment of the disease.

Requirements to applicant: recent PhD, at least one first author publication. Prior research experience with *Drosophila,* genetics, and molecular biology is advantageous. Experience in light and confocal microscopy, image processing and cell culture are helpful. The working language is English.

The Max Planck Society seeks to increase the number of women in those areas where they are underrepresented and therefore explicitly encourages women to apply.
The Max Planck Society is committed to employing more handicapped individuals and especially encourages them to apply.

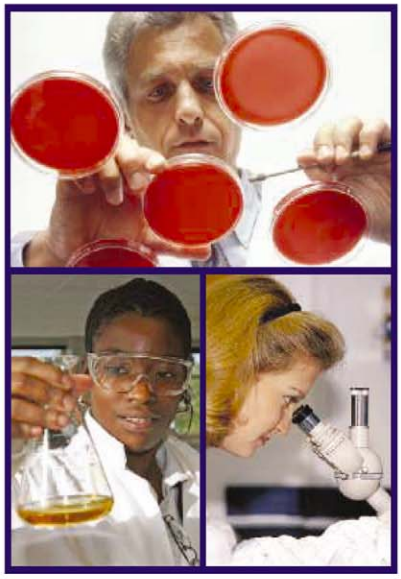
Please send your application documents as copies and not originals, as we cannot send documents back.
Applicants should send an application which includes cover letter, CV and at least two recommendation letters **preferably per e-mail** with reference to the code number to **halyna.shcherbata@mpibpc.mpg.de.**

Max-Planck-Institut für biophysikalische Chemie
Abt. Molekulare Entwicklungsbiologie
Frau Dr. Halyna Shcherbata/Code Number 16-08
Am Fassberg 11, 37077 Göttingen, Germany

Grundzertifikat 2006 audit berufundfamilie®

Food and Drug Administration

COMMISSIONER'S Fellowship Program



Protecting the Public Health Through the Science of Regulation

The FDA Commissioner's Fellowship Program

FDA is launching its new 2-year fellowship program in the fall of 2008 to provide an opportunity for health professionals and other scientists to receive training and experience at one of the world's most prestigious regulatory agencies.

You Can Make A difference

The FDA Commissioner's Fellowship Program is designed to attract top-notch physicians, microbiologists, chemists, statisticians, physicists, physiologists, pharmacists, pharmacologists, engineers, food scientists, nutritionists, veterinarians, social scientists, epidemiologists, and other scientific professionals. The fellows train minutes from the nation's Capital at FDA's new state-of-the-art campus in White Oak, Maryland or other FDA facilities of their choosing. Fellows will be exposed to the regulatory processes necessary to ensure that our drugs, biologics, food, cosmetics and devices are safe for use.

Coursework & Preceptorship

The coursework is designed to provide an in-depth review of the sciences behind regulatory review, encompassing the activities of the FDA across foods, drugs, devices and cosmetics. Coursework during the two years includes public policy, FDA law, leadership skills, epidemiology, clinical trials, statistics as well as devices and radiological health.

Under the guidance of a FDA senior scientist preceptor committed to mentoring, fellows will identify a specific aspect of FDA regulatory science to explore. Through coursework and extensive hands-on experimentation, fellows will gain in-depth knowledge of the science behind FDA regulation.

Benefits

We offer an excellent benefits package including health insurance. Candidates must have U.S. citizenship, be a non-citizen national of the U.S. or have permanent residence.

How To Apply

To apply for the FDA Commissioner's Fellowship Program, submit your curriculum vitae (CV) and identify three references with contact information to fdacommissionersfellows@fda.hhs.gov by August 29, 2008. For further information about the FDA Commissioner's Fellowship Program please visit www.fda.gov/commissionersfellowships or call 888-332-4473.

FDA is an equal opportunity employer and is a smoke-free environment.

Worcester Polytechnic Institute

ASSOCIATE PROVOST FOR RESEARCH & DEAN OF GRADUATE STUDIES

Position Description:
Worcester Polytechnic Institute (WPI) seeks nominations and applications for the position of Associate Provost for Research and Dean of Graduate Studies.

The Associate Provost for Research and Dean of Graduate Studies will be responsible for the overall operations of the Office of Research Administration, the Office of Technology Transfer, and the Office of Graduate Studies, and will report directly to the Provost.

WPI enrolls over 1,000 MS and PhD students and anticipates substantial growth in graduate studies and research.

Requirements:
The successful candidate will hold an earned doctorate and will have academic credentials and prior faculty experience appropriate for a tenured appointment at the rank of Professor.

WPI especially welcomes applications from women and underrepresented minority candidates. Applications should include a CV, a statement of vision for the position and for research and graduate education at WPI, and contact information of at least 3 professional references.

To apply please visit:
wpi.peopleadmin.com/applicants/Central?quickFind=50649

Applications will be accepted until ***September 15, 2008.***

Address inquiries to:
Search Committee Chair, c/o D.J. Graves, Office of the Provost,
100 Institute Road, Worcester, MA 01609 or djgraves@wpi.edu.

WPI is an affirmative action, equal opportunity employer.
A Member of the Colleges of Worcester Consortium

Cleveland Clinic
Lerner Research Institute

DEPARTMENT CHAIR BIOMEDICAL ENGINEERING

We are seeking a Chair for Cleveland Clinic's Department of Biomedical Engineering. The ideal applicant will have strong leadership skills and an outstanding national reputation in a research area that complements the current programs in the department. The Chair will receive a highly competitive salary, start-up support, research space and slots for new faculty. An endowed chair accompanies this position.

The Department houses 25 primary faculty (>200 total personnel) and occupies ~60,000 square feet in the Institute. The Department is one of 11 departments of Cleveland Clinic's Lerner Research Institute (LRI). Its areas of strengths include biomechanics, medical devices, biomedical imaging, BioMEMS and matrix biology/tissue engineering. The Department has strong ties to other research and clinical departments at Cleveland Clinic and nearby Case Western Reserve University.

The LRI has 160 independent investigators in its 11 departments and received >$160 million in external funding ($82 million in new funding from NIH) in 2007.

Cleveland Clinic was ranked fourth among U.S. academic medical centers in 2008 by *U.S. News and World Report*.

Send cover letter and CV to:
Guy M. Chisolm, Ph.D.
Vice Chair, Lerner Research Institute
Cleveland Clinic, NB21
9500 Euclid Avenue
Cleveland OH 44195 USA
chisolg@ccf.org

Cleveland Clinic is an Equal Opportunity Employer.

BERKELEY LAB

Deputy Chief Operating Officer

Lawrence Berkeley National Laboratory's Molecular Foundry-Opened March 24, 2006.

Berkeley Lab is a world leader in science and engineering research, with 11 Nobel Prize recipients, and 60 present members of the National Academy of Sciences. Berkeley Lab conducts unclassified research across a wide range of scientific disciplines and hosts four national user facilities. Berkeley Lab is operated by the University of California for the Department of Energy.
www.lbl.gov

Lawrence Berkeley National Laboratory is seeking a senior manager for the position of Deputy Chief Operating Officer (DCOO). Berkeley Lab conducts pioneering non-classified research across a wide range of scientific disciplines with key efforts in fundamental studies of the universe, quantitative biology, nanoscience, new energy systems and environmental solutions and the use of integrated computing as a tool for discovery.

The position provides support in coordinating and integrating the activities of all areas of Operations, including Environment, Health and Safety, Facilities, Human Resources, Information Technology, Office of the Chief Financial Officer, Office of Diversity and Ombudsman's Office and Public Affairs. The DCOO will focus on promoting effective and efficient day-to-day operations of the Laboratory and lead special projects and initiatives as assigned by the COO.

We are looking for a distinguished leader with 15+ years of management and strategic planning experience. Successful history of managing multiple projects across institutional boundaries and leading large and complex organizations is a must.

Please visit **http://jobs.lbl.gov** and enter 22030 in the search field to view the job details and apply.

Berkeley Lab is an Affirmative Action/Equal Opportunity Employer committed to the development of a diverse workforce.

Museum Director

Closing Date: 31 August 2008, or until filled

UNIVERSITY OF ALASKA MUSEUM OF THE NORTH

Responsibilities

The Director of the University of Alaska Museum of the North will be responsible for its leadership and will have primary responsibility and authority for organizing, managing, and facilitating multidisciplinary research; fundraising; budget planning and management; and personnel and public relations. The Director is spokesperson and advocate for the Museum within and outside the University, is hierarchically equivalent to deans and directors of other units within the University, and reports directly to the Provost.

Application

Applications should include a Curriculum Vitae, a letter of interest in the position, a vision statement, and the names, addresses, and phone numbers of five references. Apply at: www.uakjobs.com/applicants/Central?quickFind=62936

The review process will begin 31 August and will continue until the position is filled. The position is expected to be filled no later than 1 April 2009. Candidacy will be held in confidence until finalists are selected. More information can be found at: www.uaf.edu/museum/

Minimum Qualifications

- An advanced degree in a discipline appropriate to the Museum and appropriate for tenure in a UAF academic department.
- A substantial record of research, publication, and education in a field of natural and/or cultural history.
- Experience in museum administration, with preference given to experience in a university-affiliated museum and familiarity with the development of research collections in multiple disciplines.
- A track record of excellent management skills, with evidence of successful leadership in program building, personnel operations, fundraising, marketing, and public relations.

The successful candidate will show evidence of commitment to developing the Museum's stature and its prominent, independent role within the University of Alaska. Finalists will demonstrate an understanding and support of the Museum's tripartite mission of research, teaching, and public service and will show evidence of interest and capability to manage programs with strong interdisciplinary and international components.

UAF UNIVERSITY OF ALASKA FAIRBANKS

UAF is an AA/EEO employer and educational institution.

THE CHINESE UNIVERSITY OF HONG KONG

Applications are invited for:-

Department of Physiology

Research Assistant Professors

(Ref. 08/140(665)/2)

Applicants should have (i) a relevant PhD degree; (ii) at lease two years' postdoctoral experience; and (iii) a strong research track record in neurosciences and brain research or GI/endocrinology, in particular pancreatology. The appointees will (a) teach undergraduate and postgraduate courses; and (b) apply their expertise in one of the aforementioned areas to complement and strengthen the Department's existing research and teaching activities. Appointments will normally be made on a contract basis for up to three years initially commencing as soon as possible, leading to longer-term appointment or substantiation later subject to mutual agreement. Applications will be accepted until the posts are filled.

Salary and Fringe Benefits
Salary will be highly competitive, commensurate with qualifications and experience. The University offers a comprehensive fringe benefit package, including medical care, and a contract-end gratuity for appointments of two years or longer, plus housing benefits for eligible appointees.

Further information about the University and the general terms of service for appointments is available at *http://www.cuhk.edu.hk/personnel*. The terms mentioned herein are for reference only and are subject to revision by the University.

Application Procedure
Please send full resume, copies of academic credentials, a publication list and/or abstracts of selected published papers, together with names, addresses and fax numbers/e-mail addresses of three referees to whom applicants' consent has been given for their providing references (unless otherwise specified), to the Personnel Office, The Chinese University of Hong Kong, Shatin, Hong Kong (Fax: (852) 2603 6852). Please quote the reference number and mark 'Application - Confidential' on cover. The Personal Information Collection Statement will be provided upon request.

Vice-Chair for Research/Open Rank

The Department of Anesthesiology, University of Texas Health Science Center at San Antonio, Texas (UTHSCSA) invites nominations and applications for the position of Vice-Chair for Research (VCR). As the chief research officer for the department, the VCR is responsible for implementation of the research vision, the overall management of departmental research activities, and the administration of sponsored research. The VCR will engage in multidisciplinary collaboration within UTHSCSA—a Clinical and Translational Science Award (CTSA) grantee—and its affiliated institutions.

Qualifications for this position include an M.D., M.D.-Ph.D., or Ph.D. degree in an appropriate field of study. The successful candidate will have a national/international reputation as a distinguished scientist with an outstanding record of research accomplishments; a proven track record of directing a research enterprise; outstanding communication skills as evidenced by an ability to mentor junior faculty, scientists, residents, and students. The candidate must be a critical and strategic thinker and a visionary leader who can develop and enhance the research enterprise; and one who can demonstrate expertise in crafting interdisciplinary proposals and negotiating multi-faceted awards. One or more currently funded NIH grant(s) and experience in translational research is highly desirable. Given the excellent research infrastructure in neurobiology at UTHSCSA, research experience in pain medicine would be a plus.

For more information, please visit our website at **www.anesthesia.uthscsa.com**. To apply or nominate a candidate for the position of Vice-Chair for Research, Department of Anesthesiology, U.T. Health Science Center at San Antonio, please submit a current CV, supporting documents, and names and addresses of five references to: **J. Jeffrey Andrews, M.D, Chair, Department of Anesthesiology – MSC 7838, U.T. Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78229.**

All faculty appointments are designated as security sensitive positions. The University of Texas Health Science Center at San Antonio is an Equal Employment Opportunity/Affirmative Action Employer.

LSU Health Sciences Center

A **postdoctoral position** is available immediately in the laboratories of **Dr. Nicholas E. Goeders** in the **Department of Pharmacology, Toxicology & Neuroscience** at the LSU Health Sciences Center in Shreveport. This position will be part of a team investigating the neurobiology of stress and addiction, with special emphasis on the brain mechanisms involved in drug craving and drug seeking. Applicants should have training in behavioral pharmacology or neuroscience, and experience using in vivo microdialysis is a prerequisite for this position. Salary will be $28,000-$51,036 depending on experience. Applications should include a CV, a list of publications and the names of references familiar with the applicant's work. The LSU Health Sciences Center in Shreveport, located in northwest Louisiana, is the largest medical facility in the Tri-State area and has a reputation for excellence in medical and graduate student education and research. Excellent core facilities exist within the LSU Health Sciences Center and the adjoining Biomedical Research Institute.

Nicholas E. Goeders, Ph.D.
Professor and Head
Department of Pharmacology,
Toxicology, & Neuroscience
LSU Health Sciences Center
P.O. Box 33932
Shreveport, LA 71130-3932
NGOEDE@LSUHSC.EDU
www.sh.lsuhsc.edu

Louisiana State University is an Equal Opportunity/Affirmative Action Employer.

FACULTY POSITION
University of Pennsylvania
School of Medicine
Department of Physiology

The Department of Physiology at the University of Pennsylvania's School of Medicine seeks outstanding candidates for an Assistant Professor position on the tenure track. The successful applicant will develop a research program focused on the molecular basis of problems in physiology or cell biology. The position will also involve training and teaching graduate and/or medical students. Applicants must have an M.D., Ph.D., or equivalent degree and have demonstrated excellent research qualifications.

Please mail curriculum vitae, cover letter, three reference letters, and a statement of research interests by **August 31, 2008** to:

Physiology Search Committee
University of Pennsylvania
Department of Physiology
3700 Hamilton Walk, B400
Philadelphia, PA 19104-6085
Phys@mail.med.upenn.edu

Email submissions will not be accepted.

The University of Pennsylvania is an Equal Opportunity, Affirmative Action Employer. Women and minority candidates are strongly encouraged to apply.

INTERNATIONAL YOUNG SCIENTISTS CAREER DEVELOPMENT ORGANIZATION (ICDO) KYOTO UNIVERSITY

10 Tenure-Track Assistant Professors in the fields of life sciences

(Duration of Appointment: Nov 1, 2008 to Mar 31, 2013)

Kyoto University established International Young Scientists Career Development Organization (ICDO). The ICDO is now seeking 10 Tenure-track Assistant Professors, who are expected to focus on innovative researches in all fields of life sciences. We offer a special environment that enables young scientists to work independently. The ICDO provides an annual stipend of 6 million yen (approximately $57,000). A research grant of about 6 million yen and the stipend for one postdoctoral fellow or technician will be supplied to each ICDO Research Fellow. The application of foreign and women scientists is encouraged. Official language at ICDO will be English. Applicants are preferably under 40 years old and must have obtained a Ph.D/M.D. degree within the last 10 years. Applicants are required to submit an application form which can be downloaded from our web site. Application must reach the following address via e-mail or air mail no later than Sept 15, 2008. All correspondence should be addressed to: ICDO Administrative Office (Attn. Dr. Yoshito Kaziro), Kyoto University, Graduate School of Medicine, Yoshida Konoe-cho, Sakyo-ku, Kyoto, 606-8501, Japan. Tel:+81-75-753-9283; Fax: +81-75-753-9281; e-mail: icdo@office.med.kyoto-u.ac.jp

For more details, please visit our web site:
http://www.med.kyoto-u.ac.jp/unit/

Director of the Alaska IDeA Network of Biomedical Excellence

The University of Alaska seeks an established research scientist for the position of Principal Investigator and Director of the Alaska IDeA Network of Biomedical Excellence (INBRE). This is a senior level position at the University of Alaska Fairbanks, the lead institution for Alaska INBRE. The successful candidate must hold a doctoral degree from an accredited institution and have an established history of research funding from the National Institutes of Health that fits with the Alaska INBRE research themes focusing on emerging infectious disease, molecular toxicology, and the cellular basis of disease. This individual will collaborate with and mentor junior researchers as well as post-doctoral and graduate students. Although located on the Fairbanks campus, the Director of Alaska INBRE is responsible for planning, coordinating, and expanding biomedical research programs across the statewide university system and for interfacing with biomedical leadership in the private and public sectors in Alaska. The Director will develop and implement a vision for biomedical research programs that address basic and applied research themes, with emphasis on Alaska relevance and global importance. Together with the faculty on UA campuses, the Director will facilitate working linkages between biomedical research and education for Alaska students. Within the state, the region, and the Circumpolar North, the Director will develop strategic relationships, collaborative partnerships, and mechanisms for transfer of university technologies to the private sector. The Director has the opportunity to interact and collaborate with other biomedical and behavioral research programs in neuroscience, Alaska Native health, adaptations to high latitudes, and environmental health.

The Director of Alaska INBRE is an executive position reporting to the UAF Vice Chancellor for Research and interacting with the UA President's Subcabinet on Biomedical Research. Salary is commensurate with experience. Departmental and institute appointments for successful candidates will be available as appropriate. Information about the University of Alaska is available on our home page (http://www.alaska.edu).

For more information about this position, please contact Associate Vice Chancellor for Research John Blake at 907-474-5188 or j.blake@uaf.edu. Applications will be reviewed starting August 30, 2008 and screened until the position is filled. Visit www.uakjobs.com for complete application instructions.

THE UNIVERSITY OF ALASKA IS AN EEO/AA EMPLOYER AND EDUCATIONAL INSTITUTION.

Alaska INBRE
IDeA Network of Biomedical Research Excellence

Career Opportunities
Materials Science and Engineering

CSIRO

CSIRO is Australia's national science agency. CSIRO 's Materials Science and Engineering (CMSE) Division is the largest materials science and engineering research enterprise in Australia, employing 320 researchers in four locations across the country. The leading research of CMSE places CSIRO in the top 1% of Research Institutions in Physics, Chemistry and Engineering in the world. The Division has the twin goals of:

- Developing advanced materials that promote innovation and transform the Australian manufacturing sector, and
- Undertaking research at the frontiers of physics, chemistry and engineering to meet Australia's national challenges in water, energy and health.

It fulfils these goals in partnership with CSIRO's Flagships, national and international universities and Australian Industry.

The Division has recently employed three world leading scientists in Materials Physics (plasma nano-science), Materials Chemistry (porous inorganic materials) and Materials Science (adaptive materials) through its CEO Leadership Fellow Scheme and are seeking to employ two early career researchers and six postdoctoral fellows to work with our new Science Leaders.

For successful candidates this will be an exciting opportunity to join Australia's premier science agency. Scientists will be encouraged to develop innovative research outcomes and take part, or in some cases lead, projects of significance to the Australian Community. CSIRO pays competitive salaries with remuneration based on qualifications/experience and training.

These positions will be based in Clayton, Melbourne and in Lindfield, Sydney.

For more details on the specific roles, selection documentation and how to apply, visit **www.csiro.au/careers** then select positions vacant and select the Division CSIRO Materials Science and Engineering to see a list of all our current vacancies.

For selection documentation and details on how to apply visit **www.csiro.au/careers** or call **1300 301 509**.

hmaC086423

Children's National Medical Center

The **Center for Neuroscience Research** of the Children's Research Institute (CRI) at **Children's National Medical Center** has two tenure track openings, one each at the Full/Associate and Assistant Professor levels. These positions are for highly motivated PhD and/or MD neuroscientists with interests in the general areas of: neural stem cells and brain injury/regeneration, epilepsy, pediatric brain tumors, autism spectrum disorders, or other models of developmental disorders of the nervous system. The recruits will join a collegial and highly collaborative multidisciplinary team studying development of the CNS, with strengths in mouse genetics, neuroembryology, molecular and cellular manipulation of the developing brain, electrophysiology, confocal microscopy and time-lapse multiphoton imaging. The Center for Neuroscience Research occupies a total of 12,000 square feet of newly constructed lab space, and excellent core facilities, including transgenic and imaging cores. These positions offer competitive start-up packages. The successful candidates will have outstanding research credentials, including a substantial record of quality publications. Track record of grant support is preferred, but not required for the junior position. Each position includes a tenure track faculty appointment at the George Washington University School of Medicine.

To apply, send by **October 1, 2008** a two-page statement of research interests and goals, curriculum vitae and two-three letters of recommendation to:

Dr. Vittorio Gallo
Director, Center for Neuroscience Research
Children's Research Institute
Children's National Medical Center
111 Michigan Avenue, N.W., Ste. 5340
Washington, DC 20010-2970
Phone: 202-476-4996
E-mail: vgallo@cnmcresearch.org

Liber Ero Chair in Coastal Studies

SIMON FRASER UNIVERSITY

The Faculty of Science at Simon Fraser University invites applications for the first *Liber Ero* Chair in Coastal Studies. We are seeking an outstanding scientist with an established international reputation in an area of environmental research complementary to those of an already exceptionally strong group of researchers (Tom Buell BC Leadership Chair in Salmon Conservation, Centre for Coastal Studies, Centre for Natural Hazards Research, Centre for Wildlife Ecology, School of Resource and Environmental Management). Potential research areas include, but are not limited to, coastal ecosystem dynamics, land-ocean interactions, integrated coastal management and marine biodiversity conservation. It is anticipated that the Chair will be appointed at the rank of Associate Professor or Professor in one of the Science Departments or in the newly created Faculty of the Environment. The Chair will be expected to mount a strong and highly visible research program, to contribute to our undergraduate and graduate teaching programs and to collaborate with the Centre for Coastal Studies on outreach activities.

Research on environmental issues is a very high priority for Simon Fraser University and the Province of British Columbia, which recently created the Pacific Institute for Climate Solutions that engages the four primary research universities in the province. Given the high degree of public interest in the environment, the ability of the *Liber Ero* Chair to engage the public, relevant stakeholders and government agencies in constructive dialogue will be considered an asset.

Applicants should send a complete curriculum vitae, a concise research proposal and a list of six individuals willing to act as referees to: **Dr. Michael Plischke, Dean of Science, Simon Fraser University, 8888 University Drive, Burnaby, BC, Canada V5A 1S6; E-mail: scdean@sfu.ca.** This competition will remain open until the position is filled. Screening of applications will commence on **December 1, 2008**.

All qualified candidates are encouraged to apply; however, Canadians and Permanent Residents of Canada will be given priority. Simon Fraser University is committed to an equity employment program that includes special measures to achieve diversity among its faculty and staff. We therefore particularly encourage applications from qualified women, aboriginal Canadians, persons with disabilities and members of visible minorities.

World Class University Project

The Korean Ministry of Education, Science and Technology(MEST) is inviting excellent scholars and researchers from around the world to establish new academic projects and conduct joint research at Korean universities in the field of emerging technologies

- **What is the World Class University (WCU) project?**

The WCU project is a higher education subsidy program of the Korean government, which invites international scholars who possess advanced research capacities to collaborate with Korean faculty members and establish new academic programs in key growth generating fields.

- **Which fields does the WCU project support?**

Focus is placed on supporting new growth-generating technologies that will spearhead national development. The ministry will give priority to inter-disciplinary studies that consolidate the fields of basic sciences and humanities & social sciences which will contribute to national, social and academic development.

- **How does the WCU project work?**

Type1: Establishing new academic departments or specialized majors

Under this type, high-quality foreign scholars are employed at Korean universities as full-time faculty members, on a contract of three years minimum, to establish new academic departments or specialized majors at the universities. Undergraduate degree programs should be established and opened by the spring semester of 2010 at the latest, and graduate programs should be in operation by the fall semester of 2009 at the latest.

Type2: Recruiting foreign scholars to existing academic projects

Under this type, foreign scholars are employed as full-time faculty members at existing departments of Korean universities to conduct joint research with Korean academics. Recruited foreign scholars are expected to be capable of developing new growth-generating technologies and also creating inter-disciplinary studies.

Type3: Inviting distinguished world-class scholars

The third type invites distinguished scholars (including pioneering high-tech engineers) as part-time faculty members to conduct academic or research activities in a Korean university for a period of at least two months per academic year.

- **Who is eligible for WCU participation (foreign scholars)?**

All faculty members/researchers employed at a university, research institute or enterprise outside Korea are eligible to apply, including scholars of foreign nationality, ethnic Koreans who hold foreign nationality or citizenship, and scholars of Korean nationality.

- **What does the WCU subsidy cover?**

For types 1 and 2, the Korean government provides a competitive annual salary, research grants, and lab establishment expenses for each foreign scholar.

For type 3, the government provides an annual salary for foreign scholars and also provides research grants for joint research projects carried out with Korean scholars.

- **How can foreign scholars apply?**

All interested foreign scholars must contact Korean universities directly in order to prepare international collaboration proposals together with Korean university faculty members or research teams. Proposals should reach KOSEF by noon (12:00pm) of September 20, 2008 (Korean time), via Korean applicants.

- **Where can applicants find further details?**

Interested scholars are invited to view details and post questions about the WCU project on KOSEF's website(http://www.kosef.re.kr/english_new).

Faculty Positions
Department of Pharmaceutical Sciences
University of Hawaii at Hilo
College of Pharmacy

UNIVERSITY OF HAWAI'I HILO

The University of Hawaii at Hilo invites applicants for faculty positions in their new College of Pharmacy. This is a unique opportunity for faculty to be instrumental in supporting the development of a new college on the scenic Big Island of Hawaii. Each of the positions listed below are immediately available for full-time (11 month) appointments. The University reserves the right to hire at any rank depending on the qualifications of the selected applicants. All faculty are expected to develop an extramurally funded research program, contribute to the PharmD curriculum, serve as a faculty advisor for PharmD students, and provide service to the College and University by serving on appropriate committees. Excellent communication skills and the ability to function in a team environment are essential qualities of these positions.

Three tenure-track faculty positions are available in the Department of Pharmaceutical Sciences (Medicinal Chemistry, Pharmacology and Pharmacology/Immunology) at the Assistant, Associate or Full Professor rank. A PhD degree in the respective discipline (Medicinal Chemistry, Pharmacology or Immunology) is required or a doctorate with equivalent training in the specified area. Preference will be given to candidates who have a pharmacy background with two or more years experience in academic pharmacy. All faculty members will be expected to contribute to the scholarly pursuits of the Department in basic and applied research. It is expected that all new faculty will either have or will develop a research program that is complementary to those already existing within the Department.

The College of Pharmacy at the University of Hawaii at Hilo has attained Candidate status by the Accreditation Council on Pharmaceutical Education in 2008. The inaugural Pharm D. class was enrolled in August 2007. The review of applications will begin immediately and continue until the positions are filled. Rank and a highly competitive salary are commensurate with qualifications and experience. Interested candidates are invited to submit a letter of intent and provide their philosophy of teaching and research, a curriculum vitae, transcript and contact information for 3 professional references to the chair of the appropriate search committee. Applications will be accepted until the positions are filled.

For full details of the positions and application requirements, link to: **http://www.uhh.hawaii.edu/uhh/hr/jobs.php**

Contact Information for Pharmaceutical Sciences positions:

Ken Morris, PhD
Chair, Pharmaceutical Sciences Search Committee
University of Hawaii at Hilo – College of Pharmacy
34 Rainbow Drive
Hilo, HI 96720
(808) 933-2951
krmorris@hawaii.edu

POSITIONS OPEN

TENURE-TRACK FACULTY in MOLECULAR ONCOLOGY
The University of Michigan Medical School

The Department of Pathology is seeking tenure-track faculty to further build our research program in molecular oncology. Areas of particular interest include, but are not limited to, epigenetic regulation of transcription, high throughput functional studies (siRNA/shRNA) and protein modeling/chemical genomics.

The Department, Medical School, and Health Care System are all in excellent financial condition. Over 350 faculty actively participate in the University of Michigan Comprehensive Cancer Center. The Department operates its own graduate program and has 11 endowed chairs and over $25 million annually in research expenditures. Particular areas of research strength include molecular oncology, aging, immunology, proteomics, and informatics. The newly created Divisions of Pathology Informatics and Translational Pathology offer cutting-edge technologies in support of research programs. Planning for new building to house the Department's clinical, research, and educational activities is currently underway.

The successful applicant will hold a Ph.D. or M.D./Ph.D. and direct a vigorous research program supported by external funding. Ample resources are available to qualified applicants. Academic rank will be on the tenure-track with rank commensurate with experience. Qualified applicants should submit a letter of interest, summary of research interests, curriculum vitae, and names of three references to:

Jay L. Hess, M.D., Ph.D.
Carl V. Weller Professor and Chair
Department of Pathology
University of Michigan Medical School
Medical Science I Building, Room M5240
1301 Catherine Road
Ann Arbor, MI 48109-0602

The University of Michigan Health System is an Affirmative Action Employer and welcomes application from women and minorities.

YALE UNIVERSITY
Department of Chemistry

The Department of Chemistry at Yale University invites applications for tenure-track positions at the **ASSISTANT PROFESSOR** level to commence 1 July 2009. We seek creative teacher-scholars who show promise for developing outstanding research programs in any area of chemistry. Applicants should send their curriculum vitae and a statement of research plans, and arrange for the submission of three letters of recommendation. All materials should be received by 15 October 2008. Send applications to: **Chair, Junior Faculty Search Committee, Department of Chemistry, Yale University, P.O. Box 208107, New Haven, CT 06520-8107.** *Yale University is an Equal Opportunity/Affirmative Action Employer and applications from women and underrepresented minority group members are especially encouraged.*

A **POSTDOCTORAL FELLOWSHIP POSITION** is available in the laboratory of **Dr. Zuhair K. Ballas** at the University of Iowa. Applicants must have a Ph.D. and/or M.D. Experience in immunology and using mouse models is essential. The laboratory currently focuses on the role of NK and NKT cells in health and disease, with particular emphasis on their responses to CpG oligodeoxynucleotides and other immunomodulators using a mouse tumor model. Applications including curriculum vitae and bibliography, summary of past accomplishments, and names of three references should be sent to **Zuhair Ballas, M.D. Telephone: 319-356-3697; fax: 319-356-8280; e-mail: ballasz@uiowa.edu.**

The University of Iowa is an Equal Opportunity and Affirmative Action Employer. Women and minorities are strongly encouraged to apply.

POSITIONS OPEN

PLANT PHYSIOLOGIST TENURE-TRACK FACULTY POSITION
University of Toronto Mississauga

The University of Toronto Mississauga (UTM), Department of Biology, invites applications for a tenure-track faculty position in plant physiology at the level of **ASSISTANT PROFESSOR,** effective July 1, 2009.

We seek candidates whose research expertise addresses questions at the level of the whole organism. Subdisciplines can include global change, environmental, ecological, evolutionary, molecular, and stress physiology.

The successful applicant will have a Ph.D. and preferably postdoctoral experience, an outstanding academic record, and demonstrated excellence in research and teaching. The appointee will be expected to build an active, externally funded and internationally recognized research program and to contribute to the education and training of undergraduate and graduate students. Salary will be commensurate with qualifications and experience.

We encourage applicants to submit their application online at **website: https://utoronto.taleo.net/careersection/10050/moresearch.ftl?lang=en.** Applicants should provide curriculum vitae, statement of teaching philosophy and interests, and an outline of their proposed research. If you are unable to apply online please submit your application to: **Prof. Robert Reisz, Chair, Department of Biology, University of Toronto Mississauga, 3359 Mississauga Road North, Mississauga, Ontario, Canada L5L 1C6.** In addition, you should arrange to have three confidential letters of recommendation sent on your behalf to **e-mail: biojobs.utm@utoronto.ca.** Closing date for submissions is October 17, 2008. For more information on the Department go to **website: http://www.utm.utoronto.ca/~w3bio/homepage/.**

The University of Toronto is strongly committed to diversity within its community and especially welcomes applications from visible minority group members, women, Aboriginal persons, persons with disabilities, members of sexual minority groups, and others who may contribute to the further diversification of ideas. All qualified candidates are encouraged to apply; however, Canadians and permanent residents will be given priority.

FACULTY APPOINTMENT
Energy and Environmental Policy

The Freeman Spogli Institute for International Studies (FSI) at Stanford University seeks an outstanding scholar for a faculty appointment as a Senior Fellow who will serve as **DIRECTOR** of the Program on Energy and Sustainable Development (PESD). For some candidates there may be opportunities for joint appointment with an academic department. PESD studies the political economy of energy and environmental policy, with a particular focus on developing countries. Applicants should possess an international reputation in energy or climate change policy research as well as demonstrated ability to manage an organization. The Senior Fellow would build upon and extend into new areas the current PESD research agenda, which includes study of regulatory regimes addressing climate change, the role of national oil companies in the world oil market, global coal markets in a carbon-constrained world, and delivery of modern energy to the poor.

Qualification for a Senior Fellow position involves an academic review equivalent to the rank of Associate Professor. The appointment carries a five-year term, with the presumption of renewal. We encourage applicants from diverse disciplinary backgrounds, such as political science, economics, sociology, management science, engineering, organizational theory, law, or related social science and policy areas. Applicants should send curriculum vitae, bibliography, statement of research interest, and writing samples by October 15, 2008, to: **Belinda Byrne, Associate Director for Administration and Faculty Affairs, Freeman Spogli Institute, 616 Serra Street, Stanford, CA 94305-6055.** *Stanford University is an Affirmative Action, Equal Opportunity Employer. Applications from women and minority candidates are strongly encouraged.*

POSITIONS OPEN

ASSISTANT PROFESSOR, TENURE TRACK
Medical Biotechnology Center
University of Maryland Biotechnology Institute

The Medical Biotechnology Center (MBC) of the University of Maryland Biotechnology Institute (UMBI) seeks applications for a tenure-track faculty position at the Assistant Professor level (position #300958). The MBC is one of four research centers of the UMBI **(website: http://www.umbi.umd.edu)** and is located on the campus of the University of Maryland, Baltimore, in a newly constructed state-of-the-art research facility.

We seek outstanding candidates with expertise in extracellular matrix biology and in the use of model systems (e.g. *C. elegans*) to study basic biology. Current federal funding of the candidate's research is required. We expect broad familiarity with the following disciplines: molecular and cell biology, cellular physiology and biophysics, transgenics, or functional genomics/proteomics and skills needed to examine cellular signals at the molecular level. The ideal candidate will focus on research noted above and which broadly complements our current areas of expertise and that harmonizes with the theme of molecular signaling. The primary criteria for evaluation of candidates will be a record of excellence, originality, and productivity in research. We offer an outstanding collaborative environment with a highly competitive salary.

Applicants should send (preferably electronically to **e-mail: wrightp@umbi.umd.edu)** a letter of application (reference position # 300958), complete and current curriculum vitae, a description of research accomplishments, a two-page statement of research interests and objectives. In addition, arrange to have three letters of reference sent, preferably from two or three institutions. All materials (including letters of reference) must be received for the application to be considered. Review of applications will begin September 8, 2008, and will continue until a suitable candidate is selected.

Please send all materials to the email referenced above or by mail to**: Mr. T. Hughes, Faculty Search Committee #300958, Medical Biotechnology Center, University of Maryland Biotechnology Institute, 725 W. Lombard Street, Baltimore, MD 21201 USA.**

UMBI is an Equal Equal Opportunity/ADA/Affirmative Action Employer. Please visit us online at ***website: http://www.umbi.umd.edu.***

POSTDOCTORAL FELLOWSHIPS in IMMUNOLOGY

Postdoctoral positions are available to pursue research supported by several NIH grants including the Center of Excellence for Complementary and Alternative Medicine on Autoimmune and Inflammatory Diseases **(website: http://www.med.sc.edu/1.html).** Studies will address the effects of plant products such as resveratrol, cannabidiol and ginseng on inflammation, autoimmunity, and cancer. Ph.D. in immunology and/or molecular biology is required. Other projects include role of CD44, cannabinoids, and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on immune response. Send curriculum vitae and three references to: **Dr. Mitzi Nagarkatti, Professor and Chair, Department of Pathology, Microbiology and Immunology, University of South Carolina School of Medicine, Columbia, SC 29229** or **e-mail: postdoccam@uscmed.sc.edu.** *USC Columbia is an Equal Opportunity Affirmative Action Employer and encourages applications from women and minorities.*

POSTDOCTORAL FELLOW POSITION

Postdoctoral position at the University of Alabama at Birmingham, to study the effects of nicotine exposure on kidney disease. Ph.D. in biochemistry, physiology, pharmacology, or molecular biology is required. Experience in kidney disease is desirable but not necessary. E-mail curriculum vitae and summary of research experience to **Edgar A. Jaimes, M.D.** at **e-mail: tmp-ejaimes@mail.ad.uab.edu.**

POSITIONS OPEN

TWO POSTDOCTORAL POSITIONS
Cancer Molecular Biology with Clinical Translation

Two Postdoctoral positions are available at the University of Colorado at Denver Health Sciences Center for talented and dedicated scientists to join a multidisciplinary team committed to clinically translating novel epidermal growth factor receptor–family activities in breast cancer.

Project one will focus on the emerging field of receptor tyrosine kinase (RTK) nuclear signaling. We have identified an RTK that regulates therapeutic response and functions as both a transcriptional coactivator and a BH3-only protein. The successful candidate will employ gene expression and promoter arrays, mouse models, and breast cancer patient samples to elucidate the molecular mechanisms of RTK nuclear signaling contributing to breast cancer. This candidate will have a strong background in molecular biology with experience performing chromatin immunoprecipitation a must and experience in epigenetics and/or analysis of mouse phenotypes a plus.

Project two involves the characterization of a novel oncogene in breast cancer that promotes therapeutic resistance, alters miRNA expression, and silences tumor suppressors. The interested candidate will develop a diagnostic assay and decipher the molecular basis of therapeutic evasion mediated by this oncogene. This candidate will have a strong background in molecular biology with experience in TaqMan assay development and epigenetic analysis a must and experience in the analysis of xenograft models, miRNA activity, and/or cellular signaling pathways a plus.

Both candidates must be highly motivated and have a strong desire to clinically translate their findings. Interested applicants with a Ph.D., M.D., or M.D./Ph.D. degree should send their curriculum vitae, description of research accomplishments and interests, and contact information for three professional references to: **Dr. Frank Jones, e-mail: frank.jones@uchsc.edu.** Further information about the **Jones** laboratory can be found at **website: http://www.uchsc.edu/pathology/joneslab/.**

ASSISTANT PROFESSOR/ASSOCIATE PROFESSOR, VETERINARY PARASITOLOGY
Department of Veterinary Science
Louisiana State University

An Assistant Professor/Associate Professor position is available in the area of parasitology. The major focus of the position will be to establish a nationally recognized and externally funded research program that will lead to improvement of existing or development of new control strategies for parasites of livestock. The candidate must develop and obtain research grants while conducting original and innovative research. Applicant should have a D.V.M. and/or Ph.D., postdoctoral experience, and evidence of research accomplishments. Creative, energetic investigators with a broad background in parasitology and research programs and experience in the immunologic, genetic, molecular, cellular, and/or biochemical aspects of the discipline are particularly encouraged to apply. The Louisiana State University (LSU) AgCenter offers a diverse and highly collaborative research environment with state-of-the-art equipment available. In addition the Department of Veterinary Science has close ties to the LSU School of Veterinary Medicine. Interested applicants should submit a letter of application that includes a statement of research goals and curriculum vitae with three references to:

Dr. Thomas R. Klei
Chair of the Search Committee
Louisiana State University
Department of Veterinary Science
111 Dalrymple Building, Baton Rouge, LA 70803
Telephone: 225-578-9727
Fax: 225-578-4890
E-mail: klei@vetmed.lsu.edu

For more information visit **website: http://www.lsuagcenter.com.**

LSU is an Equal Opportunity/Affirmative Action Employer.

POSITIONS OPEN

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

INSTRUCTOR of MEDICINE POSITION

The University of Alabama at Birmingham, Division of Cardiovascular Disease is seeking applicants with a Ph.D. or M.D. for the position of Instructor. This is a nontenure-earning faculty position. Candidates must be capable of independently designing and conducting experiments and preparing and submitting data for publication. It is expected that the successful candidate will be able to secure independent extramural funding to support ongoing research. Individuals interested in this position should have experience in small animal surgery and be able to work in both in vitro and in vivo. Interested candidates should send curriculum vitae, three letters of reference, and a brief statement of your interest to: **Robert C. Bourge, M.D., Director, 1900 University Boulevard, University of Alabama at Birmingham Station, Birmingham, AL 35294.**

University of Alabama at Birmingham is an Equal Opportunity/Affirmative Action Employer.

POSTDOCTORAL RESEARCH FELLOW
University of Calgary, Faculty of Veterinary Medicine, Alberta, Canada
Dr. Christoph Muelling
Department of Comparative Biology and Experimental Medicine
Website: http://www.vet.ucalgary.ca/faculty/members/dr_christoph_mulling

Dr. C. Muelling has an opening for a Postdoctoral Research Fellow in his research program at the University of Calgary. Dr. Muelling's research focuses on the pathogenesis of equine and bovine lameness as well as expression of prion protein in deer antler velvet (additional details on website). A Ph.D. in veterinary medicine or other appropriate area as well as hands-on knowledge of histological, molecular, and cellular biology techniques is essential. At least one research project as evidenced by a first author paper published or accepted in a peer-reviewed journal is also required.

This is two-year appointment with the opportunity for renewal. Competitive salary and benefits are available. Candidates should submit a cover letter, curriculum vitae, and three references, via e-mail to: **Dr. C. Muelling; Professor of Veterinary Anatomy; Assistant Dean, Curriculum; Faculty of Veterinary Medicine; University of Calgary; e-mail: ckw.mueling@ucalgary.ca.**

The M.D. Anderson Cancer Center is recruiting an experienced Flow Cytometry Specialist to be **COORDINATOR** of the Flow Cytometry Laboratory at the Science Park Research Division. The primary responsibilities of this position are operation and maintenance of a Becton Dickinson Aria. The successful candidate will have excellent interpersonal and communication skills in order to assist the multidisciplinary faculty and trainees in choosing fluorochromes, optimizing experimental parameters, and interpreting data.

Additional job requirements are: a Bachelor's or more advanced degree in biological science; a minimum of two years of experience in operating a flow cytometer; and computer proficiency using Mac and PC platforms.

For information about Science Park see **website: http://sciencepark.mdanderson.org.**

Interested candidates should send curriculum vitae and names of three references to:

Ellen Richie, Ph.D.
Department of Carcinogenesis
Director, Cell and Tissue Analytical Core
E-mail: bbrooks@mdanderson.org

University of Texas MDACC is an Equal Opportunity/Affirmative Action Employer and a smokefree workplace.

CONFERENCE

POSITIONS OPEN

The Department of Biochemistry at Brandeis University invites applications for a tenure-track faculty position at the rank of **ASSISTANT PROFESSOR** beginning fall 2009. We are searching for a creative scientist who will establish a vigorous independent research program and who also demonstrates a commitment to teaching. The research program should address fundamental questions concerning biological and/or biochemical function at the molecular level. Research fields of interest include, but are not limited to, nucleic acid biochemistry, chemical biology, and biochemistry of complex systems.

Brandeis offers the rare combination of an internationally recognized research university in a small college setting. The suburban campus is just 20 minutes from Boston and Cambridge, and is part of the vibrant community of academic and biotechnology centers in the Boston area.

Electronic submission of applications is preferred. Candidates should submit their curriculum vitae, a brief research proposal, and three letters of reference to **e-mail: lolsen@brandeis.edu** or by regular mail to:

Dagmar Ringe, Chair Search Committee
c/o Lynn Olsen
Department of Biochemistry
MS013
P.O. Box 549110
Waltham, MA 02454-9110

First consideration will be given to applications received by November 1, 2008.

Brandeis University is an Equal Opportunity Employer, committed to building a culturally diverse intellectual community, and strongly encourages applications from women and minority candidates.

ECOLOGIST (MODELING)

The Department of Biology seeks a broadly trained Ecologist for a full-time, tenure-track **ASSISTANT PROFESSOR** position to begin July 1, 2009. The successful candidate will approach central questions in ecology from a computational or modeling perspective and use such techniques as key components of an active research program involving undergraduates. Teaching responsibilities will include participation in introductory biology, a general course in computational biology, and advanced undergraduate courses in ecology and ecological modeling. This position will contribute to the College's interdepartmental Program in Environmental Studies.

A Doctorate and at least one year of postdoctoral experience are required. Submit curriculum vitae, a description of research plans that addresses the role of undergraduates in your research, a statement of teaching philosophy that includes areas of teaching interests, and arrange for three letters of recommendation to be sent by September 24, 2008, to: **Chair, Ecology Search, Department of Biology, Bryn Mawr College, 101 N. Merion Avenue, Bryn Mawr, PA 19010-2899.** Electronic submissions **(e-mail: jjacoby@brynmawr.edu)** must be accompanied by a hard copy of all materials for full consideration.

Located in suburban Philadelphia, Bryn Mawr College is a highly selective liberal arts college for women who share an intense intellectual commitment, a self-directed and purposeful vision of their lives, and a desire to make meaningful contributions to the world. Bryn Mawr comprises an undergraduate college with 1,200 students, as well as coeducational graduate schools in some humanities, sciences, and social work. The College participates in a consortium together with Haverford and Swarthmore Colleges and the University of Pennsylvania. *Bryn Mawr College is an Equal Opportunity, Affirmative Action Employer. Minority candidates and women are especially encouraged to apply.*

POSITIONS OPEN

POSTDOCTORAL POSITION
NEURO-ONCOLOGIC NEUROSURGERY
Thomas Jefferson University/Jefferson Medical College

The Department of Neurological Surgery at Thomas Jefferson University is seeking a motivated individual to investigate vaccine candidates in animal glioma models as part of a multidisciplinary group studying brain tumor immunity in patients and in animal models.

The successful candidate will possess a Ph.D. in immunology with an interest in neuroimmunity and/or tumor vaccine development as well as expertise in the analysis of T cell function.

Interested individuals should forward their curriculum vitae, along with a list of three references, to:

David W. Andrews, M.D., FACS
E-mail: david.andrews@jefferson.edu
D. Craig Hooper, Ph.D.
E-mail: douglas.hooper@jefferson.edu
909 Walnut Street, Third Floor
Philadelphia, PA 19107
Telephone: 215-503-1774
Fax: 215-923-7745

Thomas Jefferson University/Jefferson Medical College is an Affirmative Action/Equal Opportunity Employer. Women and people of diverse racial, ethnic, and cultural backgrounds are encouraged to apply.

ASSISTANT/ASSOCIATE PROFESSOR
The Ohio State University
College of Medicine
Division of Cardiothoracic Surgery

The Division of Cardiothoracic Surgery in the Department of Surgery at the Ohio State University College of Medicine seeks a qualified candidate for a tenure-track faculty member at the Assistant/Associate Professor level. Salary and academic rank will be based on experience and qualifications. The candidate must have a Ph.D./M.D. degree and should have three or more years of postdoctoral experience. Candidates with currently funded extramural grant(s) are preferred.

The candidate is expected to have an independent research program oriented towards cardiovascular biomedical engineering. Candidates with strong research interest and ongoing programs on blood vessel development, heart remodeling, biomaterials, assist devices, and tissue engineering may apply with a copy of resume and a brief description of research interest to: **Benjamin Sun, M.D., Chief of Ohio State University Cardiothoracic Surgery, N-847 Doan Hall, 410 W. 10th Avenue, Columbus, OH 43210.** *The Ohio State University is an Equal Opportunity/Affirmative Action Employer. Qualified women, minorities, Vietnam-era veterans, disabled veterans, and individuals with disabilities are encouraged to apply.*

A **POSTDOCTORAL POSITION** in bioinspired materials engineering is available in a leading multidisciplinary collaborative research group, which is combining traditional developmental biology and molecular cell biology approaches with cutting-edge tools from chemistry, materials science, physics, and engineering to develop entirely new approaches to organ engineering and cancer therapy. The project focuses on development of bioinspired tissue scaffolds that mimic the way in which embryonic tissues influence tissue pattern formation, as well as tumor differentiation. Ph.D. or M.D./Ph.D. candidates should have experience in polymer chemistry, biomolecular self assembly, or genetic engineering of biopolymers, preferably with an interest or expertise in tissue engineering. Background in biopolymer synthesis and characterization, as well as surface chemistry modification, is also preferred.

Send applications to: **Donald Ingber, M.D., Ph.D., Vascular Biology Program, Departments of Pathology and Surgery, Children's Hospital/Harvard Medical School, 300 Longwood Avenue, KFRL 11.127, Boston, MA 02115. E-mail: jeanne.nisbet@childrens.harvard.edu.**

Children's Hospital is an Equal Opportunity Employer that places strong emphasis on the values of quality, equality, diversity, and compassion.

POSITIONS OPEN

POSTDOCTORAL/RESEARCH ASSOCIATE in REPRODUCTIVE ENDOCRINOLOGY
Women's Health and Infant Development Research Center, Eastern Virginia Medical School

Postdoctoral research opening for Ph.D. or M.D. is available in the newly established Women's Health and Infant Development Research Center at Eastern Virginia Medical School (EVMS) to study fetal ovarian development in the baboon. Training background in basic research in reproductive endocrinology, experience in molecular biology, and *U.S. citizenship* are required. Candidates should submit letter of interest and curriculum vitae via e-mail to: **Sandra Huband, Women's Health and Infant Development Research Center, Eastern Virginia Medical School, Norfolk, VA 23507; e-mail: hubandsb@evms.edu.**

MARKETPLACE